EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

31 January 2024
EMA/HMPC/240553/2016
Committee on Herbal Medicinal Products (HMPC)

Assessment report on *Foeniculum vulgare* Miller subsp. *vulgare* var. *vulgare,* fructus *and Foeniculum vulgare* Miller subsp. *vulgare* var. *dulce* (Mill.) Batt. & Trab., fructus

Final – Revision 1

Based on Article 16d(1), Article 16f and Article 16h of Directive 2001/83/EC (traditional use)

Herbal substance(s) (binomial scientific name of the plant, including plant part)		*Foeniculum vulgare* Miller subsp. *vulgare* var. *vulgare,* fructus *Foeniculum vulgare* Miller subsp. *vulgare* var. *dulce* (Mill.) Batt. & Trab., fructus
Herbal preparation(s)		*Foeniculum vulgare* Miller subsp. *vulgare* var. *vulgare*: dry fruit *Foeniculum vulgare* Miller subsp. *Vulgare* var. *dulce* (Miller) Batt. & Trab.: dry fruit
Pharmaceutical form(s)		Herbal substance as herbal tea for oral use.
First assessment	Rapporteur(s)	M. Delbò
Revision	Rapporteur(s)	A. Assisi
	Peer-reviewer	W. Dymowski

Official address Domenico Scarlattilaan 6 ● 1083 HS Amsterdam ● The Netherlands
Address for visits and deliveries Refer to www.ema.europa.eu/how-to-find-us
Send us a question Go to www.ema.europa.eu/contact **Telephone** +31 (0)88 781 6000 An agency of the European Union

Table of contents

1. Introduction

1.1. Description of the herbal substance(s), herbal preparation(s) or combinations thereof

This assessment report revises and updates the set of data used in the first HMPC assessment report to support the establishment of individual European Union herbal monographs and/or European Union list entries on bitter fennel fruit and sweet fennel fruit.

- Herbal substance(s)

Foeniculum vulgare Mill. subsp. *vulgare* belongs to the *Apiaceae* (Umbelliferae) botanical family.

The European Pharmacopoeia (Ph. Eur.) describes two varieties: sweet (var. *dulce*) and bitter fennel fruit (var. *vulgare*)

Sweet fennel fruit consists of dry cremocarps and mericarps of *Foeniculum vulgare* Mill. subsp. *vulgare* var. *dulce* (Mill.) Batt. & Trab., and it is characterised by a content of essential oil not lower than 20 mL per kg anhydrous fruit with a 80.0% minimum content of anethole in its essential oil. Sweet fennel is pale green or pale yellowish-brown (Ph. Eur. 10th Edition 04/2011:0825 corrected 10.0).

Bitter fennel fruit consists of dry cremocarps and mericarps of *Foeniculum vulgare* Mill. subsp. *vulgare* var. *vulgare,* and it contains not less than 40 mL per kg anhydrous fruit of essential oil that contains not less than 60.0% of anethole and not less than 15.0% of fenchone. Bitter fennel is greenish-brown, brown or green (Ph. Eur. 10th Edition 04/2013:0824 corrected 10.0).

The fruit is administered after crushing, in solid or liquid dosages*,* sweet fennel being more broadly used (Niesel, 1992).

The medicinal properties of fennel fruit are mainly attributed to its content of essential oil, whose main constituent is *trans*-anethole.

The fennel fruits also contain water-soluble glycosides of monoterpenoid, alkyl and aromatic compounds (Kitajima *et al*., 1998a; Kitajima *et al*., 1998b; Kitajima *et al*., 1998c), as well as, among other substances, proteins, cellulose, lignin, pectins, triglycerides containing mainly petrosilinic, oleic and linoleic acids, wax esters, phospholipids, phytosterols (e.g., beta-sitosterol and stigmasterol), flavonoids, hydroxycoumarins, furanocoumarins and vitamins (tocopherol and tocotrienol) (Kunzemann and Herrmann, 1977; Zlatanov, 1994; Ivanov and Aitzetmuller, 1995; Reiter and Brandt, 1985; Council of Europe, 2002).

Some chemotypes are known for their lower content of trans-anethole (less than 50%), higher content of fenchone (more than 30%) and higher content of estragole (more than 30%) (Teuscher *et al*., 2005).

A considerable variability among relative proportions of different compounds in fennel fruits has been observed in relation to the methodology used for extraction (Diaz-Maroto *et al*., 2005).

Methanolic extract from Tunisian (TTS) and French fennel seeds (FFS) showed significant differences in total phenolic content; the French seed extracts (bitter fennel) had lower amounts of phenolic contents than Tunisian seeds (sweet fennel). The phenolic acids represented the major classes of phenols in both TTS and FSS extracts, with quinic acid present in all methanolic extracts, whilst TFS have relatively higher amounts of 4-*O*-caffeoylquinic acid, *p*-coumaric acid, and 3,4-di-*O*-caffeoyquinic acid compared to FSS. The flavonoid contents of TFS were higher than that of FFS (Kalleli *et al.*, 2019).

- Herbal preparation(s)

Comminuted or powdered fennel fruits

Sweet and bitter fennel fruits are used mainly crushed as herbal teas. Powdered sweet fennel fruit is also used. Crushed or powdered fennel fruits gradually lose their volatile constituents upon aging (Czygan, 1989). Teabags examined 30 days after opening showed in general a loss of essential oil ranging from 4 to 10%. Moreover, in these samples a decrease of anethole content and an increase of anisaldehyde content (considered as the degradation product of the former) was also evident (Bilia *et al*., 2002).

Chemical compositions of volatiles in infusions or microwave decoctions prepared from crushed fruits or in teas from pre-packaged tea bags or in instant teas may be very different among themselves and from volatiles obtained by hydro-distillation of crushed fruits (Forster, 1983; Bilia *et al*., 2002). Anethole (30-90%) and/or anisaldehyde (0.7-51.0%) were detected in all the samples; estragole (0.8-4.1%), eugenol (1.5-11.3%) and fenchone (0.5-47.0%) were detected in most samples (Bilia *et al*., 2002).

Quantification of estragole content in commercial fennel herbal teas with an analytical method based on Stir Bar Sorptive Extraction and GC–MS was carried out in order to allow for a more accurate estimate of the dietary exposure to estragole. Concentration levels ranged from 241 to 2058 µg/L in teas from teabags, from 9 to 912 µg/L in diluted instant teas, from 251 to 1718 µg/L in teas from not packaged seeds. Based on these data and considering the daily consumption of three portions of herbal tea, a maximum exposure to estragole for adults of 10 µg/kg bw per day was calculated. Some authors concluded that the relatively high level observed in diluted instant teas of some brands deserves attention since these products are designed for infant consumption. According to the same authors, estimated exposure in infants is up to 51 µg/kg bw per day for teas from teabags, and up to 23 µg/kg bw per day for instant teas. Findings suggested that a generalisation of the use of suitable technologies in production processes of instant teas could substantially reduce the exposure to estragole in the vulnerable population groups (infants, young children, pregnant and breastfeeding women) who consume fennel products (Raffo *et al*., 2011).

Fennel tea products of 42 different brands (tea bags, n=20; dry fennel fruits, n=19; instant teas, n=3) were collected on the Austrian market. Three brands of fennel fruits and one brand of tea bags contained bitter fennel. For all other brands, no information on the variety was available. Fennel teas were prepared as recommended on the respective label of the product to reflect the usual consumers' preparation at home. A modified GC-MS method was applied to determine the estragole level in the tea infusions. Average estragole contents were 565 µg/L in infusions prepared from tea bags and 639 µg/L in teas prepared from dry fennel fruits. Maximum estragole concentrations were 2477 µg/L and 4644 µg/L. Instant teas on the Austrian market did not contain estragole. Consumption data of different population groups in Austria were obtained from a survey conducted within the scope of the Austrian Nutrition Report 2008; an average exposure was assessed using a tea consumption of 95.4 mL, 194.7 mL, and 114 mL for children, women, and men, respectively. In a kind of worst case scenario, a high tea consumption (95th percentile) of 363 mL for children, 1000 mL for women, and 600 mL for men was considered. For the exposure assessment of infants (aged 0–12 months) and toddlers (aged 1–3 years), a default body weight of 5 kg and 12 kg, respectively, was assumed based on EFSA (2012). The estimated daily exposure from consumption of fennel teas ranged from 0.25 to 5.04 µg/kg per day, 0.32 to 6.42 µg/kg day, and 0.15 to 2.93 µg/kg day for children, women, and men, respectively. Daily estragole exposures for infants were 0.008–20.78 µg/kg per day. Despite margin of exposure (MOE) values are above 10,000 for nearly half of the fennel teas analysed, there are still MOEs below

this value indicating a potential risk for human health and a priority for risk management. (Mihats *et al.*, 2016).

The German Chemisches und Veterinäruntersuchungsamt (CVUA, 2007) tested anise and fennel teas (EMA/HMPC/137212/2005 Rev 1 Corr 1*). The extraction efficiency was less than 2%. A recent study of van den Berg *et al*. (2014) described the analysis of estragole content in dry fennel preparations and in infusions prepared from them with a special emphasis on extraction efficiency. The range of estragole levels was 0.15-13.3 mg/g in starting dry fennel preparations, whereas the estragole content in infusions was considerably lower ranging between 0.4 and 133.4 µg/25 mL infusion prepared from 1 g dry material (i.e., 0.016-5.34 µg/mL). Extraction efficiency varied between <0.1 to 2.5% in a sample of 37 fennel-based preparations.

Also the nature of the starting material was important because infusions prepared from whole fennel fruits contained about three-fold less estragole compared to infusions prepared from fine cut fennel material. In general, extraction efficiencies depend on many variable factors and the best estimate for any product or process is probably reached by extraction experiments with the preparation itself (EMA/HMPC/137212/2005 Rev 1 Corr 1*).

- Combinations of herbal substance(s) and/or herbal preparation(s) including a description of vitamin(s) and/or mineral(s) as ingredients of traditional combination herbal medicinal products assessed, where applicable.

Not applicable.

1.2. Search and assessment methodology

This assessment report reviews the available scientific data for bitter fennel and sweet fennel (i.e., *Foeniculum vulgare* Miller sp. *vulgare* var. *Vulgare,* fructus and *Foeniculum vulgare* Miller sp. *vulgare* var. *dulce* (Miller) Batt. & Trab., fructus, respectively) and particularly clinical data.

In preparing this assessment report, medical databases have been reviewed. The results of a data search carried out in October 2020 in Embase, Medline, Pubmed covering the period from year 2011 to 2020 were taken into consideration in the revision process of the monographs.

Search engines used: Google Scholar

Toxicological databases: Toxnet

Pharmacovigilance resources: The results of a data search on Eudravigilance carried out in May 2021.

2. Data on medicinal use

2.1. Information about products on the market

2.1.1. Information about products on the market in the EU/EEA Member States

Information on medicinal products marketed in the EU/EEA

Table 1: Overview of data obtained from marketed medicinal products: Foeniculi amari fructus

Active substance	Indication	Pharmaceutical form Strength (where relevant) Posology Duration of use	Regulatory Status (date, Member State)
Foeniculi amari fructus	Traditional herbal medicinal product for symptomatic treatment of mild, spasmodic gastro-intestinal complaints including bloating and flatulence (before 2013 Dyspeptic complaints such as mild, spasmodic gastro-intestinal ailments, anorexia, bloating and flatulence). Effect on hormones such as stimulating milk production. Catarrh of the upper respiratory tract, cough (expectorant)	Herbal tea 2 g per tea bag, oral; adolescents and adults 2 g x 3 daily; children 4-12 years: 2 g x 2 daily; children 6 months-4 years: 2 g x 1-2 daily	Since 2013 THMP according to Directive 2001/83/EC Austria
Foeniculi amari fructus	a) Symptomatic treatment of mild, spasmodic gastro-intestinal complaints, bloating and flatulence. b) Catarrh of the upper respiratory tract	Herbal tea for oral use Single dose: 2.5 g Daily dose: 5-7.5 g Can be used in infants and toddlers for preparation of baby food or for dilution of milk	1986, Germany WEU
Foeniculi amari fructus	a) Traditionally in symptomatic treatment of mild gastro-intestinal disturbances as feeling of fullness and bloating b) Symptomatic treatment of mild spasmodic complaints (as in menstruation) c) As an expectorant in cough connected with common cold	Infuse one 2 g teabag of whole seeds in 250 mL of boiling water for 15 min. Use in adults and adolescents (over 12 years): 3 times a day drink a fresh-prepared infusion. Use in adolescents is accepted in mild gastro-intestinal disturbances, in the same dosage as in adults, for no longer than 2 weeks. In children in the age of	Koper włoski fix Authorised since 05.06.1992, PL. Currently TUR

Active substance	Indication	Pharmaceutical form Strength (where relevant) Posology Duration of use	Regulatory Status (date, Member State)
		4-12 years: 3 times a day half of a glass (100–125 mL) of the infusion. Duration of use: for no longer than 1 week	
Foeniculi amari fructus	THMP used in: a) mild spasmodic complaints symptomatic treatment of mild spasmodic gastro-intestinal complaints as feeling of fullness, bloating and flatulence; b) symptomatic treatment of mild spasmodic complaints (as in menstruation); c) as an expectorant in cough connected with common cold	One 2 g of whole seeds sachet pour with 250 mL (one glass) of boiling water, keep under cover for 15 min. Adults and adolescents: drink 3 times a day. In mild spasmodic complaints with bloating and flatulence, in adolescents the same dose as for adults. In children in age 4-12 years, 3 times 100 mL (half of glass) 3 times a day. Daily dose for adults and adolescents 4.5-7.5 g; in children 3–5 g	Owoc kopru włoskiego 1997, PL
Foeniculi amari fructus	THMP used in: a) mild spasmodic complaints symptomatic treatment of mild spasmodic gastro-intestinal complaints as feeling of fullness, bloating and flatulence; b) symptomatic treatment of mild spasmodic complaints (as in menstruation); c) as an expectorant in cough connected with common cold	As above	Owoc kopru włoskiego. Authorised since 01.06.1997, PL. Currently TUR
Foeniculi amari fructus		Loose fruits, in bag	Owoc kopru włoskiego 1998, PL

Active substance	Indication	Pharmaceutical form Strength (where relevant) Posology Duration of use	Regulatory Status (date, Member State)
Foeniculi amari fructus		Infusion bags (sachets)	Owoc kopru włoskiego 1998
Foeniculi fructus	Medicinal product used in symptomatic treatment of mild spastic gastrointestinal complaints, including bloating and flatulence	Infusion bags (sachets) 2 g of whole seeds. 1 sachet pour with a glass of boiling water and infuse 15 min. under cover. Adults and adolescents 1 glass of the infusion 3 times a day. Children 4-12 years half glass of the infusion 3 times a day	Owoc kopru włoskiego 2002.09, PL

Table 2: Overview of data obtained from marketed medicinal products: Foeniculi dulci fructus

Active substance	Indication	Pharmaceutical form Strength (where relevant) Posology Duration of use	Regulatory Status (date, Member State)
Foeniculi dulcis fructus	a) for symptomatic treatment of mild, spasmodic gastro-intestinal complaints including bloating, and flatulence b) as an expectorant in cough associated with cold	Herbal tea for oral use Adolescents and adults:1 tea bag (1.5 g)/250 mL of boiling water 3 times daily Children 4–12 years: 1 tea bag (1.5 g)/250 mL of boiling water 2-3 times daily Duration of use: Adults and adolescents: not to be taken for more than 2 weeks. Children 4 - 12 years: for short-term use in mild transitory symptoms only (less than one week)	On the market since 2000 Czech Republik Switched to the traditional herbal medicinal product 13.4.2011 TU
Foeniculi dulcis fructus, powdered	a) THMP for symptomatic treatment of mild, spasmodic gastro-intestinal	Capsules containing 300 mg. Posology for adults and adolescents: 3 capsules before lunch	Since 1989, Spain Registered according former national registration scheme.

Active substance	Indication	Pharmaceutical form Strength (where relevant) Posology Duration of use	Regulatory Status (date, Member State)
	complaints including bloating, and flatulence b) THMP for symptomatic treatment of minor spasm associated with menstrual periods c) THMP used as an expectorant in cough associated with cold	and 3 capsules before dinner. Duration of use: 2 weeks	In May 2011, the product was registered according to Article 16c of Directive 2001/83/EC
Foeniculi dulcis fructus	symptomatic treatment of digestive upsets such as epigastric distension, slow digestion, eructation, flatulence	Herbal tea for oral use 1.8 g 2 to 3 times daily	February 1990, France TU Not more marketed at least since 2011
Foeniculi dulcis fructus (original information; currently there are no monocomponent medicinal products containing fennel authorised/registered in Latvia)	a) as a carminative, for gastrointestinal disorders and spasms b) as galactagogue increases breast-milk production c) as a mild expectorant d) For children: for colic and as carminative (ATC code: V03 AX)	Herbal tea for oral use Daily dosage: 5.0-7.0 g crude drug or equivalent preparations as an infusion. Infusion: pour 180 mL of hot water over a tablespoon (~ 5.0 g) of the fruits, allow them to stand for 20 min., then remove the fruits with a strainer. Half cup of the freshly prepared infusion is drunk 2-3 times daily before eating. For children aged up to 4 years daily dose is 1 teaspoon, for children aged 4 -10 years: 1 dessertspoon of the fruits	Since 1970 marketed in Latvia until 2010 as a medicinal product. Still on the market as a food supplement. Fructus Foeniculi, species

Active substance	Indication	Pharmaceutical form Strength (where relevant) Posology Duration of use	Regulatory Status (date, Member State)
Foeniculi dulcis fructus, powdered	a) symptomatic treatment of digestive upsets such as: epigastric distension, slow digestion, eructation, flatulence b) as an adjuvant treatment for the painful component of functional digestive disorders	Hard capsules 390 mg 3 times a day (if necessary: until 1,950 mg daily)	since November 1990, France TU Not more marketed at least since 2011

This overview is not exhaustive. It is provided for information only and reflects the situation at the time when it was established.

Information on relevant combination medicinal products marketed in the EU/EEA

Various fixed combinations containing fennel (as herbal tea, oral drops) are authorised/registered in different European countries, mainly with indications related to mild gastrointestinal disorders associated with bloating, flatulence or mild spasm or as an adjuvant for treatment of acute and chronic upper respiratory tract disorders associated with cough or catarrhs.

Information on other products marketed in the EU/EEA (where relevant)

Food supplements containing fennel fruit are on the market in different Member States.

Fennel essential oil maybe used as flavouring agent in cosmetics (IE).

2.1.2. Information on products on the market outside the EU/EEA

See section 2.2 on Traditional Chinese Medicine (TCM).

2.2. Information on documented medicinal use and historical data from literature

The treaty "Farmacologia Teorica e Pratica", also named "Farmacopea Italiana" of Giuseppe Orosi (1851- Vincenzo Mansi Ed.-Livorno) lists fennel fruit in the Materia Medica Botanica Chapter (Orosi, 1851).

Anti-asthma and dyspnea effects have been described for fennel in Iranian ancient medical books (Avesina, 1985). Applications in the treatment of catarrh of the upper respiratory tract has been described in several handbooks and treaties (Brand, 1993; Czygan, 1989; Madaus, 1976; Parfitt, 1993; Merkes, 1980; Weiss, 1997; and Müller-Limmroth and Fröhlich, 1980).

Fennel fruit has also been reported as useful in the treatment of dyspeptic complaints such as mild, spasmodic gastro-intestinal ailments, bloating and flatulence (Brand *et al*., 1993; Czygan, 1989; Madaus, 1976; Schilcher, 1984 and 1986).

Fennel fruit has been reported to be in use in some areas for many years to relieve painful menstruation, symptoms of female climacteric and other purposes (Hare *et al*., 1916; Albert-Puleo, 1980; Zargari, 1992; Mills *et al*., 2000; Jahromi *et al*., 2003).

Fennel fruit (Xiaohuixiang) has been in use for many centuries in Chinese Medicine, generally as decoction with other herbs (L'Italia Agricola, 1989, Chinese Herbal Medicine 1999, p. 203-204) and the Chinese Herbal Medicine (1999, p. 445, 503) classifies fennel fruit as being able "to control gastrointestinal smooth muscle" and to treat "stomach ache". Fennel fruit (Xiaohuixiang) is described in the Pharmacopoeia of the People's Republic of China (English edition, 2005, Vol. I;) with the action: "to dispel cold and relieve pain, to regulate the stomach function". The indications reported in the Chinese Pharmacopoeia are as follows: "it is used for lower abdominal pain with cold sensation, dysmenorrhoea; distending pain in the epigastrium with reduced appetite, vomiting and diarrhoea; hydrocele testis".

For oral use in TCM the dosage is 3-6 g per day as a herbal tea in single or divided doses.

According to the TCM, fennel fruit can be 'hotted' and wrapped in a bag (10 grams) for ironing the lower abdomen to treat abdominal pain of cold type (Liu Gan Zhong *et al*., 2003).

It was recommended for bronchitis and chronic coughs, kidney stones, dysmenorrhea, vomiting and diarrhoea, and defection of sperm. It was considered to have diuretic, stomachic and galactogogue properties due to its volatile compounds (He & Huang, 2011).

Fennel has been used as lactagogue since antiquity with no side effects reported (Keller, 1992).

Other uses described in traditional medicines include treatment of blepharitis, bronchitis, constipation, conjunctivitis, diabetes, diarrhoea, dyspnoea, fever, gastritis, headache, pain, poor appetite and respiratory and urinary tract infections. As an aphrodisiac, anthelminthic, emmenagogue, galactagogue and vermicide (WHO, 2010).

Table 3: Overview of historical data

Note: where no specification on the variety is given in the literature, the reference is considered for both sweet and bitter fennel fruit. In this case the variety is mentioned in brackets.

Herbal preparation	**Documented Use / Traditional Use**	**Pharmaceutical form** **Strength (where relevant)** **Posology** **Duration of use**	**Reference**
Foeniculi amari fructus	Dyspepsia such as mild, spastic gastrointestinal affliction, fullness and flatulence. For catarrh of the upper respiratory tract	Adult and children over 12 years: daily dose 5-7 g of crushed or ground seeds for teas; 10-20 g of fennel syrup or honey; 5–7.5 g of fennel tincture (=5-7.5 mL). Equivalent preparations: infusion: 1-3 g in 150 mL water, 2 -3 times daily between meals; fluid extract 1:1 (g/mL): 1-3 mL, 2-3 times between meals; tincture 1:5 (g/mL): 5-15 mL, 2 to 3 times daily between meals; native dry	Blumenthal and Goldberg, 2000

Herbal preparation	**Documented Use / Traditional Use**	**Pharmaceutical form** **Strength (where relevant)** **Posology** **Duration of use**	**Reference**
		extract 3.9-4.9:1 (w/w): 0.2-0.7 g, 2-3 times daily between meals	
Foeniculi amari fructus	Standardzulassung: a) In gastrointestinal complaints, flatulence b) As an expectorant of the airways	Standardzulassung: 2.5-7.5 g freshly crushed fruits in 150 mL boiling water as an infusion (infusion time 5-10 min) between meals 2-4 times daily	Czygan, 1989
Foeniculi amari fructus	a) Gastrointestinal complaints, chronic constipation with bloating flatulence, flatulence with colic, diarrhoea and gastrointestinal cramps b) As expectorant in bronchitis, cough, also in varicose and whooping cough, lung disease c) as galactagogue, (emmenagogue, diuretic) d) as an eye wash for strengthening eyes in asthenopia and conjunctivitis e) flu, rachitis, scrofula	5.5-7.4 g daily as an infusion	Madaus 1976
Foeniculi (dulcis and amari) fructus	As a carminative and expectorant	2-3 g for infusion	Keller 1992
Foeniculi (dulcis and amari) fructus	To relief gastrointestinal complaints	Powdered:1-4 g Infusion 3%: one cup after each meal	Leclerc 1983
Foeniculi (dulcis and amari) fructus	Abdominal bloating; lack of appetite; slow digestion; aerophagia; gastric pain; nervous vomiting; intestinal parasites; lung disturbances; prevention of flu; oliguria (small volume of urine) and bladder stones; gout; insufficient menstrual flow; milk insufficiency in breastfeeding women	Powdered:1-4 g daily Infusion 1 coffee spoon for a cup (10 min. of infusion): one cup after each meal Flu prevention: chew fennel fruits	Valnet 1990
Foeniculi (dulcis and amari) fructus	Flatulent dyspepsia. Anorexia. Flatulent colic in children. Topically: conjunctivitis. Blepharitis (eye wash). Pharyngitis (gargle)	0.3-0.6 g or as infusion thrice a day Liquid extract: 1:1 in alcohol 70% V/V. 0.8-2 mL thrice a day Aq. Foenic. Conc. B.P.C. (1034) 0.31-1 mL thrice a day	British Herbal Pharmacopoeia 1983

Herbal preparation	Documented Use / Traditional Use	Pharmaceutical form Strength (where relevant) Posology Duration of use	Reference
		Aq. Foenic. Dest. B.P.C. (1934) 15-30 mL thrice a day	

2.3. Overall conclusions on medicinal use

Table 4: Overview of evidence on period of medicinal use: Foeniculi amari fructus

Herbal preparation Pharmaceutical form	Indication	Strength Posology	Period of medicinal use
Foeniculi amari fructus Herbal tea	Traditional herbal medicinal product for symptomatic treatment of mild, spasmodic gastro-intestinal complaints including bloating and flatulence	*Adolescents and adults:* 2 g x 3 daily; *Children 4-12 years*: 2 g x 2 daily; *Children 6 months-4 years*: 2 g x 1-2 daily.	since 2013 THMP according to Directive 2001/83/EC Austria
Foeniculi amari fructus Herbal tea	a) Symptomatic treatment of mild, spasmodic gastro-intestinal complaints, bloating and flatulence. b) Catarrh of the upper respiratory tract	Single dose: 2.5 g Daily dose: 5-7.5g Can be used in infants and toddlers for preparation of baby food or for dilution of milk	1986, Germany WEU
Foeniculi amari fructus Herbal tea	THMP used in: a) mild spasmodic complaints symptomatic treatment of mild spasmodic gastro-intestinal complaints as feeling of fullness, bloating and flatulence; b) symptomatic treatment of mild spasmodic complaints (as in menstruation); c) as an expectorant in cough connected with common cold	One 2 g of whole seeds sachet pour with 250 mL (one glass) of boiling water, keep under cover for 15 min. Adults and adolescents: drink 3 times a day. In mild spasmodic complaints with bloating and flatulence, in adolescents the same dose as for adults. In children in age 4-12 years, 3 times 100 mL (half of glass) 3 times a day. Daily dose for adults and adolescents 4.5-7.5 g; in children 3–5 g	Several herbal medicinal products in Poland, since 1992 Currently TUR
Foeniculi amari fructus Herbal tea	Standardzulassung: a) In gastrointestinal complaints, flatulence b) As an expectorant of the airways	Standardzulassung: 2.5-7.5 g freshly crushed fruits in 150 mL boiling water	Czygan, 1989

Herbal preparation Pharmaceutical form	Indication	Strength Posology	Period of medicinal use
		as an infusion (infusion time 5-10 min) between meals 2-4 times daily	
Foeniculi amari fructus Herbal tea	a) Gastrointestinal complaints, chronic constipation with bloating flatulence, flatulence with colic, diarrhoea and gastrointestinal cramps b) As expectorant in bronchitis, cough, also in varicose and whooping cough, lung disease	5.5-7.4 g daily as an infusion	Madaus 1976

Long-standing use for at least 30 years, 15 of them within the European community, is therefore demonstrated for dried bitter fennel whole or (freshly) comminuted (also crushed) fruit as a herbal tea for the following indications:

a) for symptomatic treatment of mild, spasmodic gastro-intestinal complaints including bloating and flatulence. Traditional use for this indication is substantiated by the presence on the market in Germany since 1986, in Poland for more than 30 years, and the presence in a number of papers (Madaus, 1976; Leclerc 1983; Valnet, 1990; Czygan, 1989). It is also used in children. (Poland, Austria, Madaus, 1976);

b) as an expectorant in cough associated with cold. Traditional use for this indication is substantiated by the presence on the market in Germany since 1986 and in Poland since 1992. It is also mentioned in Madaus 1976;

c) for symptomatic treatment of minor spasm associated with menstrual periods. Traditional use in this indication is substantiated by the presence on the market in Poland since 1992.

Posology for indications a), b) and c) (based on long-standing use):

Adults and adolescents

Single dose

1.5-2.5 g of comminuted fruits with 0.25 l of boiling water for 15 min. 3 times daily as a herbal tea.

Daily dose: 4.5–7.5 g

Children under 12 years of age (indications a) and b) only)

Single dose

1.0 g-1.7 g of comminuted fruits with 0.25 L of boiling water for 15 min., 1-3 times a day, as a herbal tea. For short-term use in mild transitory symptoms only (less than one week).

Daily dose: 3.0–5.0 g

The posologies above reported are supported by evidence of long-standing use based on products on the market and bibliographic references. Due to new data available from literature on the genotoxicity and carcinogenicity of estragole, recently the HMPC has revised the "Public statement on the use of herbal medicinal products containing estragole" (EMA/HMPC/137212/2005 Rev 1 Corr 1*), concluding that:

1. "…the intake of estragole from HMPs in the general population should be as low as possible, which includes a short-time duration of use (maximum 14 days) and a discussion about the single/daily doses necessary for adults and adolescents according to the risk assessment relevant for the concerned HMP. For example, to reach or come as close as possible to the guidance value of 0.05 mg/person per day, the lowest dose should be consistently selected if ranges of single and daily doses are available from traditional use".

2. "The usage of estragole containing HMPs in children is not recommended if the daily intake of estragole exceeds the guidance value of 1.0 µg/kg bw, unless otherwise justified by a risk assessment based on adequate safety data."

Therefore, in case of adults and adolescents, only the lower dose of 1.5 g of fruits with 0.25 L of boiling water three times daily as a herbal tea will be included in the monograph. This corresponds to a daily dose of 4.5 g.

Similarly, for children between 4-12 years of age, only the lower dose of 1.0 g fruits with 0.25 L of boiling water 3 times daily as a herbal tea has been included in the monograph. This corresponds to a daily dose of 3.0 g.

However, the use is not recommended if the daily intake of estragole exceeds the guidance value of 1.0 µg/kg bw, unless otherwise justified by a risk assessment based on adequate safety data.

The use is not recommended in children under four years of age due to the lack of adequate data.

Raffo *et al*. (2011) demonstrated a 5.9 and 4.4-fold increase of the estragole content in infusion prepared from freshly broken fruits in comparison to those prepared from intact fennel fruits. Also, Mihats *et al*. (2016) found the highest content of estragole in an infusion prepared using dried crushed fennel fruits. Therefore, to minimise the exposure to estragole, the use of comminuted fruits has been taken out from the monograph.

Ph. Eur. requires min. 40 mL/kg (3.8-3.9%) of essential oil in bitter fennel fruit and min. 20 mL/kg (1.9-1.95%) in sweet fennel fruit. The content of essential oil in fennel fruits present on the market is variable.

The determined oil yields ranged between 22.2 and 50.7 mL/kg in dried fennel fruits obtained from pharmacies in EE, NO, AT and Moldova. For sweet fennel, essential oil content was in the range 22.2–32.0 mL/kg, whereas for bitter fennel it was 50.5–50.7 mL/kg (Raal *et al.*, 2012).

On the Austrian general market, 42 different herbal tea brands of bitter fennel was found to contain 3–8.5% essential oil, while sweet fennel exhibits significantly less essential oil (0.8–3%) (Mihats *et al.*, 2016).

Essential oil yield of slightly crushed, maturated fruit from fennel samples in Turkey were between 3.5-10.3 mL/100 g (Telci *et al.*, 2019). No information provided on the variety (*dulce* or *vulgare*).

The extraction rates of estragole from fennel fruit to infusions, depend on many variable factors and the best estimate for any product or process is probably reached by extraction experiments with the preparation itself. Extraction rates reported in literature are highly variable.

Raffo *et al.* (2011) found the extraction efficiency of fennel essential oil into the infusion of 25–35% as used by the ESCO working group (EFSA, 2009a) relatively high, leading to an overestimation of the estragole content in the infusion.

Zeller and Rychlik (2006) experimentally determined an extraction efficiency of 12% for estragole from crushed fennel seeds into an infusion.

Recently, Van den Berg *et al.* (2014) determined an extraction efficiency of estragole from dry fennel preparations into the infusion equal to 0.1–2.3%.

Based on the content of essential oil in bitter fennel fruits, the extraction rates of estragole from fennel fruits to infusions reported in literature, and on the limits on the content of estragole in the essential oil of bitter fennel fruits set by the relevant Ph. Eur. monograph, even with low doses of 3 g per day of herbal tea, the intake of estragole in children aged between 4-11 years might be above the guidance value of 1.0 µg/kg bw set by the HMPC in the "Public statement on the use of herbal medicinal products containing estragole" (EMA/HMPC/137212/2005 Rev 1 Corr 1*). Therefore, without prejudice to the obligation to comply with the quality requirements established by the Ph. Eur. for fennel substance and preparations which are legally binding, each action from cultivation of the plant to the manufacture of herbal medicinal product, which could minimise the exposure of humans to estragole is recommended.

Duration of use

Adults and adolescents

Not to be taken for more than 2 weeks.

Children between 4-12 years of age (indications a) and b) only
For short-term use in mild transitory symptoms only (less than one week).

If the symptoms persist during the use of the medicinal product, a doctor or a qualified health care practitioner should be consulted.

Table 5: Overview of evidence on period of medicinal use: Foeniculi dulcis fructus

Herbal preparation Pharmaceutical form	**Indication**	**Strength Posology**	**Period of medicinal use**
Foeniculi dulcis fructus Herbal tea	a) for symptomatic treatment of mild, spasmodic gastro-intestinal complaints including bloating, and flatulence b) as an expectorant in cough associated with cold	*Adolescents and adults*:1 tea bag (1.5 g)/250 mL of boiling water 3 times daily *Children 4–12 years*: 1 tea bag (1.5 g)/250 mL of boiling water 2-3 times daily Duration of use: *Adults and adolescents:* not to be taken for more than 2 weeks. *Children 4-12 years*: for short-term use in mild transitory symptoms only (less than one week)	On the market since 2000 Czech Republic Switched to the traditional herbal medicinal product 13.4.2011 TU

Herbal preparation Pharmaceutical form	Indication	Strength Posology	Period of medicinal use
Foeniculi dulcis fructus, comminuted capsules (containing 300 mg)	a) THMP for symptomatic treatment of mild, spasmodic gastro-intestinal complaints including bloating, and flatulence b) THMP for symptomatic treatment of minor spasm associated with menstrual periods c) THMP used as an expectorant in cough associated with cold	*Adults and adolescents*: 900 mg before lunch and 900 mg before dinner. Duration of use: 2 weeks	Since 1989, Spain Registered according former national registration scheme. In May 2011, the product was registered according to Article 16c of Directive 2001/83/EC
Foeniculi dulcis fructus	symptomatic treatment of digestive upsets such as epigastric distension, slow digestion, eructation, flatulence	Herbal tea for oral use 1.8 g, 2-3 times daily	February 1990, France TU Not more marketed at least since 2011
Foeniculi dulcis fructus Herbal tea	a) as a carminative, for gastrointestinal disorders and spasms b) as galactagogue increases breast-milk production c) as a mild expectorant d) For children: for colic and as carminative (ATC code: V03 AX)	Daily dose: 5.0-7.0 g crude drug or equivalent preparations as an infusion. Infusion: pour 180 mL of hot water over a tablespoon (~ 5.0 g) of the fruits, allow them to stand for 20 min., then remove the fruits with a strainer. Half cup of the freshly prepared infusion is drunk 2-3 times daily before eating. For children aged up to 4 years daily dose is 1 teaspoon, for children aged 4-10 years:1 dessertspoon of the fruits	Since 1970 marketed in Latvia until 2010 as a medicinal product. Currently there are no monocomponent medicinal products containing fennel authorised/registered in Latvia. Still on the market as a food supplement
Foeniculi dulcis fructus, powdered Hard capsules	a) symptomatic treatment of digestive upsets such as: epigastric distension, slow digestion, eructation, flatulence b) as an adjuvant treatment for the painful component of functional digestive disorders	390 mg 3 times a day (if necessary: until 1,950 mg daily)	Since November 1990, France TU Not more marketed since 2011.

Long-standing use for at least 30 years, 15 of them within the European community, is therefore demonstrated for sweet fennel whole or (freshly) comminuted (also crushed) fruit as a herbal tea and for powdered fruit to be taken orally in capsules for the following indications:

a) for symptomatic treatment of mild, spasmodic gastro-intestinal complaints including bloating, and flatulence. Traditional use for this indication is substantiated by the presence on the market in France and Latvia for more than 30 years. It is registered as a THMP according Article 16 c) of Directive 2001/83/EC in Czech Republic. It is also used in children (Latvia and Czech Republic).

b) as an expectorant in cough associated with cold. Traditional use for this indication is substantiated by the presence on the market in Latvia, Spain, France since more than 30 years, and the registration as a THMP according Article 16 c) of Directive 2001/83/EC in Czech Republic.

c) for symptomatic treatment of minor spasm associated with menstrual periods. Traditional use in this indication is supported based on the presence on the market in Spain since 1989.

Comminuted fruit as herbal tea: posology (based on long-standing use)

Adults and adolescents

Single dose
1.5-2.5 g comminuted fennel fruits with 0.25 L of boiling water (brew for 15 min.) three times daily as a herbal tea.

Daily dose: 4.5–7.5 g
Children under 12 years of age (indications a) and b) only)

Average daily dose:
3-5 g of (freshly) comminuted fruits as a herbal tea, in 3 divided doses, for short-term use in mild transitory symptoms only (less than one week).

For adults and adolescents, taken into considerations the conclusion of the HMPC in the "Public statement on the use of herbal medicinal products containing estragole" (EMA/HMPC/137212/2005 Rev 1 Corr 1*) and in analogy with bitter fennel fruits, only the lower dose of 1.5 g of fruits with 0.25 L of boiling water three times daily as a herbal tea is included in the monograph. This corresponds to a daily dose of 4.5 g.

Similarly, for children between 4-12 years of age, only the lower dose of 1.0 g of fruits with 0.25 L of boiling water three times daily as a herbal tea will be included in the monograph. This corresponds to a daily dose of 3.0 g.

However, the use is not recommended if the daily intake of estragole exceeds the guidance value of 1.0 µg/kg bw, unless otherwise justified by a risk assessment based on adequate safety data.

The use is not recommended in children under four years of age due to the lack of adequate data.

Raffo *et al.* (2011) demonstrated a 5.9 and 4.4-fold increase of the estragole content in infusion prepared from freshly broken fruits in comparison to those prepared from intact fennel fruits. Also, Mihats *et al.* (2016) found the highest content of estragole in an infusion prepared using dried crushed fennel fruits. Therefore, to minimise the exposure to estragole, the use of comminuted fruits has been taken out from the monograph.

Based on the content of essential oil in sweet fennel fruits, the extraction rates of estragole from fennel fruits to infusions reported in literature, and on the limits on the content of estragole in the essential oil of sweet fennel fruits set by the relevant Ph. Eur. monograph, even with low doses of 3 g per day of herbal tea, the intake of estragole in children aged between 4-11 years might be above the guidance value of 1.0 µg/kg bw set by the HMPC in the "Public statement on the use of herbal medicinal products containing estragole" (EMA/HMPC/137212/2005 Rev 1 Corr 1*). Therefore, without prejudice to the obligation to comply with the quality requirements established by the Ph. Eur. for fennel

substance and preparations, each action from cultivation of the plant to the manufacture of herbal medicinal product which could minimise the exposure of humans to estragole should is recommended.

Duration of use:

Adults and adolescents
Not to be taken for more than 2 weeks.

Children between 4-12 years of age (indication a) and b) only)
For short-term use in mild transitory symptoms only (less than one week).

If the symptoms persist during the use of the medicinal product, a doctor or a qualified health care practitioner should be consulted.

Powdered sweet fennel fruits to be taken orally in capsules: posology (based on long-standing use)

Adults and adolescents

900 mg (3 capsules x 300 mg), 2 times daily; daily dose: 1.8 g.

The intake of estragole with the powdered sweet fennel fruits according to the above reported posology is expected to be much higher if compared to the intake which would be assumed with the herbal teas according to the posologies supported by the evidence of traditional use for the same therapeutic indications. It has been demonstrated that crushing fennel seeds increases the release of estragole (Raffo *et al.*, 2011; Mihats *et al.*, 2016). In addition, in the case of the powdered sweet fennel fruits taken orally in capsules, the content of estragole is not reduced by the extraction process during the preparation of the infusion. Therefore, the use of powdered sweet fennel fruits is not included in the monograph.

3. Non-Clinical Data

3.1. Overview of available pharmacological data regarding the herbal substance(s), herbal preparation(s) and relevant constituents thereof

3.1.1. Primary pharmacodynamics

- *Effects of fennel on gastro-intestinal tract*

Addition of 0.5% of powdered fennel seeds to the diet of rats for 6 weeks shortened food transit time by 12% ($p<0.05$) (Platel and Srinivasan, 2001).

Fennel administered orally at 24 mg/kg bw increased spontaneous movement of the stomach in unanaesthetised rabbits and reduced the inhibition of stomach movement induced by sodium pentobarbitone (Niiho *et al*., 1977).

Fennel aqueous extracts, in a concentration of 10% weight/volume, perfused through the stomach of anaesthetised rats at 0.15 mL/min. and collected over periods of 20 min. increased gastric acid secretion in the stomach of anesthetised rats from the basal level of 0.12 to 0.42 mL ($p<0.02$). It has been shown that fennel increases gastric acid secretion. An experiment showed that following administration of a fennel aqueous extract, the increase in acid production in acetylsalicylic acid-injured stomachs compared to that in healthy stomachs was markedly reduced, leading to the conclusion that gastric stimulation requires a healthy, intact gastric mucosa (Vasudevan *et al*., 2000).

Fennel water suspension of powdered fennel fruits (250 mg/kg and 500 mg/kg bw) administered intraperitoneally and orally to Wistar albino rats showed a dose-dependent ulcer protective effects in different gastric ulcer models induced by pyloric ligation (Shay), hypothermic restraint stress, indomethacin and by necrotizing agents (80% ethanol, 0.2 M NaOH and 25% NaCl). Pre-treatment with fennel suspension reversed the depletion of gastric wall mucus, replenished the decrease in non-protein sulfhydryls and contrasted the reduction of MDA levels induced by 80% ethanol (Al-Mofleh *et al*., 2013).

Oral administration of anethole (0.3 mg/kg and 3 mg/kg) significantly improved clonidine-induced delayed gastric emptying examined by the phenol red method. Anethole was administered to mice (fasted for 18 hours) and clonidine 30 mg/kg was given by subcutaneous administration 50 min. later. Anethole also stimulated gastric accommodation in rats (Asano *et al*., 2016).

- *Spasmolytic effect on contracted smooth muscles*

Fennel fruit alcoholic extracts and oil exerted a relaxing effect on *in vitro* pre-contracted smooth muscles from different organs (tracheal, ileal and uterine) by antagonizing several contraction-inducing agents.

A 30%-ethanolic extract from bitter fennel (1 part of drug to 3.5 part of ethanol 31% w/w) produced a concentration-dependent decrease in acetylcholine-and histamine-induced contractility of isolated guinea pig ileum at concentrations of 2.5-10 mL/L; however, taking into account the effect of ethanol, only the results with histamine were significant ($p<0.005$ at 10 mL/L) (Forster et al., 1980). In the same test system, the extract at 2.5 and 10 mL/L also concentration-dependently reduced carbachol-induced contractility (Forster, 1983).

Addition of anethole to 10 to 25 mL/L of physiological solution in which an isolated mouse intestinal jejunum is plunged induced intestinal motility at low concentrations, but an intestinal relaxation was observed at concentrations higher than 50 mg/L (Imaseki & Kitabatake, 1962).

- *Anti-inflammatory effect*

The 70% ethanol extract and a water extract of air-dried and chopped fennel fruits considerably inhibited 5-lipoxigenase-catalysed leukotriene production from A23187-induced rat basophilic leukemia (RBL)-1 cells. The IC_{50} was 3.2 µg/mL and 25.4 µg/mL for ethanolic and water extract, respectively. Among the constituents isolated from the extract, several terpene derivatives including γ-terpinene and fenchone as well as phenylpropanoid, trans-anethole, showed considerable inhibitory action of 5-lipoxigenase. In particular, the IC_{50} of *trans*-anethole was 51.6 mM. In addition, the ethanolic extract (100 mg/kg) and *trans*-anethole (50 mg/kg and 200 mg/kg) showed significant inhibition by oral administration against arachidonic acid-induced ear oedema in mice (Lee *et al*., 2012).

Oral pre-treatment of rats with a dry 80%-ethanolic extract from sweet fennel at 100 mg/kg bw inhibited carrageenan-induced paw oedema by 36% ($p<0.01$) compared to 45% inhibition by indometacin at 5 mg/kg (Mascolo *et al*., 1987).

The anti-inflammatory effects of fennel (herbal preparation not reported) was investigated in model of lipopolysaccharide (LPS)-induced acute lung injury. In five groups, the mice were intraperitoneally injected with 1% Tween 80-saline (vehicle), fennel (125, 250, 500 µl/kg), or dexamethasone (DEX) (1 mg/kg), followed 1 h later by intratracheal instillation of LPS (1.5 mg/kg). In the remaining two groups, the mice were intraperitoneally injected with 1% Tween 80-saline (vehicle) or fennel (250 µl/kg), followed 1 h later by intratracheal instillation of sterile saline. Fennel significantly and dose-dependently reduced lactate dehydrogenase (LDH) activity and immune cell numbers in LPS treated

mice. In addition fennel effectively suppressed the LPS-induced increases in the production of the inflammatory cytokines interleukin-6 and tumour necrosis factor-alpha, with 500 µl/kg fennel showing maximal reduction. Fennel also significantly and dose-dependently reduced the activity of the proinflammatory mediator matrix metalloproteinase 9 and the immune modulator nitric oxide (Lee *et al*., 2015).

Trans-anethole was administrated (36.4, 72.8 or 145.6 mg/kg) as well as dexamethasone (5 mg/kg) orally once daily for 7 consecutive days in mice with acute lung injury induced by LPS (24 mg/kg). *Trans*-anethole, as well as dexamethasone, eliminated LPS-induced histopathological changes, decreased the number of inflammatory cells and resulted in a notable reduction in IL-17 mRNA expression. In addition, trans-anethole increased IL-10 mRNA expression in isolated lung tissues and resulted in a marked elevation in T regulatory cells and reduction in T helper 17 cells in spleen tissues (Zhang *et al*., 2018).

- *Secretolytic and expectorant effects*

An increase of about 12% in mucociliary transport velocity was observed in isolated ciliated epithelium from the frog oesophagus 90 sec. after application of 200 µl of an infusion from bitter fennel (4.6 g per 100 mL of water) (Müller-Limmroth and Fröhlich, 1980).

Anethole and fenchone vapour were given by inhalation to urethanised rabbits as doses of 1 to 243 mg/kg bw added to the steam vaporizer (the amount actually absorbed by the animals being considerably less, estimated as not more than 1% of that added to the vaporizer). Inhalation of anethole did not affect the volume but produced a dose-dependent (1-9 mg/kg) decrease in the specific gravity of respiratory tract fluid. Inhalation of fenchone produced a dose-dependent (1-9 mg/kg) augmentation of the volume output of respiratory tract fluid and a dose-dependent (1-27 mg/kg) decline in its specific gravity (Boyd and Sheppard, 1971).

Table 6: Overview of the main non-clinical data/conclusions

Herbal preparation tested	**Strength** **Dosage** **Route of administration**	**Experimental model** ***In vivo*/** ***In vitro***	**Reference** **Year of publication**	**Main non-clinical conclusions**
Effects on gastro-intestinal tract				
Powdered fennel seeds	0.5% of the diet for 6 weeks	*In vivo* rats	Platel and Srinivasan, 2001	shortened food transit time by 12% ($p<0.05$)
Fennel (no other information available)	orally at 24 mg/kg bw	*In vivo* rabbits	Niiho *et al*., 1977	increased spontaneous movement of the stomach in and reduced the inhibition of stomach movement induced by sodium pentobarbitone
Fennel aqueous extract (10% w/v) from roasted seeds	0.15 mL/min.	*In vivo* rats perfusion through the stomach and collection over periods of 20 min.	Vasudevan *et al.*, 2000	gastric acid secretion significantly increased ($p<0.02$) to more than 3-fold compared to the basal secretion determined from perfusion of saline solution. The increase in acid production in acetylsalicylic acid-injured stomachs compared to that in healthy stomachs was markedly reduced, leading to the conclusion that gastric stimulation requires a healthy, intact gastric mucosa
Fennel water suspension of powdered fennel fruits	Intraperitoneally or orally at 250 mg/kg and 500 mg/kg bw	*In vivo* rats	Al-Mofleh *et al*., 2013	dose-dependent ulcer protective effects; pre-treatment reversed the depletion of gastric wall mucus, replenished the decrease in non-protein sulfhydryls and contrasted the reduction of MDA levels induced by 80% ethanol
Anethole	Orally at 0.3 mg/kg and 3 mg/kg	*In vivo* rats	Asano *et al*. 2016	significantly improved clonidine-induced delayed gastric emptying and stimulated gastric accomodation
Spasmolytic effect on contracted smooth muscles				
30% ethanolic extract from bitter fennel (1 part	2.5 and 10 mL/L	*In vitro* isolated guinea pig ileum	Forster *et al*., 1980	The extract produced a concentration-dependent decrease in acetylcholine-and histamine-induced contractility of isolated

Herbal preparation tested	**Strength** **Dosage** **Route of administration**	**Experimental model** ***In vivo*/** ***In vitro***	**Reference** **Year of publication**	**Main non-clinical conclusions**
of drug to 3.5 part of ethanol 31% w/w)		a) +2.5 µg/L acetylcholine b) +10240 µg/L histamine		guinea pig ileum at concentrations of 2.5-10 mL/L; however, taking into account the effect of ethanol, only the results with histamine were significant ($p<0.005$ at 10 mL/L)
30% ethanolic extract from bitter fennel (1 part of drug to 3.5 part of ethanol 31% w/w)	2.5 and 10 mL	*In vitro* isolated guinea pig ileum + carbachol	Forster *et al*., 1983	The extract produced a concentration-dependent decrease in carbachol induced contractility of isolated guinea pig ileum at concentrations of 2.5-10 mL/L
single substances: anethole	10 to 25 mL/L of physiological solution	*In vitro* isolated mouse intestinal jejunum	Imaseki *et al*., 1962	induced intestinal motility at low concentrations, but an intestinal relaxation was observed at concentrations higher than 50 mg/L
Secretolytic and expectorant effects				
infusion from bitter fennel (4.6 g per 100 mL of water)	200 µl	*In vitro* isolated ciliated epithelium from the frog oesophagus	Müller-Limmroth and Fröhlich, 1980	90 sec. after application increase of about 12% in mucociliary transport velocity
Anethole vapour	1 to 243 mg/kg bw added to the steam vaporizer by inhalation (the amount actually absorbed by the animals being considerably less, estimated as not more than 1% of that added to the vaporizer)	*In vivo* urethanised rabbits	Boyd and Sheppard, 1971	Inhalation of anethole did not affect the volume but produced a dose-dependent (1-9 mg/kg) decrease in the specific gravity of respiratory tract fluid

Herbal preparation tested	**Strength** **Dosage** **Route of administration**	**Experimental model** ***In vivo*/** ***In vitro***	**Reference** **Year of publication**	**Main non-clinical conclusions**
Fenchone vapour	1 to 243 mg/kg bw added to the steam vaporizer by inhalation (the amount actually absorbed by the animals being considerably less, estimated as not more than 1% of that added to the vaporizer)	*In vivo* urethanised rabbits	Boyd and Sheppard, 1971	Inhalation of fenchone produced a dose-dependent (1-9 mg/kg) augmentation of the volume output of respiratory tract fluid and a dose-dependent (1-27 mg/kg) decline in its specific gravity
Anti-inflammatory effects				
70% ethanol extract of air-dried and chopped fennel fruits; *trans*-anethole	Ethanol extract orally at 100 mg/kg *Trans*-anethole orally at 50 and 200 mg/kg	*In vivo* mice	Lee *et al*., 2012	Significant inhibition against arachidonic acid-induced ear oedema in mice
dry 80%-ethanolic extract from powdered sweet fennel	100 mg/kg bw orally	*In vivo* rats carrageenan-induced paw oedema	Mascolo *et al*., 1987	Oral pre-treatment inhibited carrageenan-induced paw oedema by 36% ($p<0.01$) compared to 45% inhibition by indometacin at 5 mg/kg
Fennel (details on herbal preparation not reported)	Intraperitoneally at 125, 250, 500 µl/kg	*In vivo* Mice: model of LPS-induced acute lung injury	Lee *et al*. 2015	significant and dose-dependent reduction of LDH activity and immune cell numbers in LPS treated mice; suppression of the LPS-induced increases in the production of IL-6 and TNF-α; significant and dose-dependent reduction of the activity of MMP-9 and nitric oxide
Trans-anethole	Orally at 36.4, 72.8 or 145.6 mg/kg per 7 days	*In vivo* Mice: model of LPS-induced acute lung injury	Zhang *et al*., 2018	Eliminated LPS-induced histopathological changes, decreased the number of inflammatory cells, reduced IL-17 mRNA expression, increased IL-10 mRNA expression, increase T regulatory cells and reduced T helper 17 cells in spleen tissues

3.1.2. Secondary pharmacodynamics

- *Antimicrobial effects*

Fennel fruit extracts exhibited *in vitro* strong inhibitory activities against the growth of a wide spectrum of bacteria and fungi known to be pathogenic for man and other species.

Acetone, n-butanol, ethanol and ether extracts of fennel inhibited the growth of a range of bacteria including *Escherichia coli* and *Staphylococcus aureus*, and also exhibited antifungal activity against *Candida albicans* and other organisms (Maruzzella & Freundlich, 1959).

Staphylococcus aureus isolates in food revealed the highest significant level of susceptibility against the ethanolic extract of fennel fruits compared with other studied bacteria ($p>0.05$); on the other hand, Salmonella enteric isolates in food revealed the highest significant level of resistance to the ethanolic extract of fennel compared with other studied bacteria ($p>0.05$). *Escherichia coli* and *Bacillus cereus* bacteria isolates revealed similar level of susceptibility against the ethanolic extract of fennel. The standard strains of *Escherichia coli*, *Salmonella enteric*, *Staphylococcus aureus* and *Bacillus cereus* revealed highest to lowest level of resistance to the ethanolic extract of fennel, respectively (Mahdavi *et al.*, 2017).

Chatterjee *et al.* (2016) have demonstrated that a methanol extract of sweet fennel seeds significantly inhibited cholera toxin production in various *Vibrio cholerae* strains, regardless of serogroup or biotype. Interestingly, transanethole and 4-allylanisole, essential oil components of sweet fennel seeds, also demonstrated similar effects (Chatterjee *et al.*, 2016).

The antimicrobial potential of supercritical CO_2 fluid extract obtained from powdered *Cichorium intybus*, *Cinnamomum camphora*, *Commiphora myrrha*, *Foeniculum vulgare*, *Nerium oleander*, and *Spartium junceum* plants was determined using agar well diffusion method through the formation of clear zones against 10 pathogenic test microorganisms including both Gram-positive (MRSA clinical isolate, *Enterococcus faecalis*, *Streptococcus mutans*, *Micrococcus* sp.) and Gram-negative (*Enterobacter cloacae*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Salmonella typhimurium*) bacteria as well as yeasts (*Candida albicans* and *Candida lipolytica*). Crude extract of *Foeniculum vulgare* showed the highest potency against *Candida albicans*, *Candida lipolytica, Enterococcus faecalis,* and *Salmonella typhimurium*, with inhibition zones of 14, 15, 22 and 20 mm, respectively (Sulemain 2020).

- *Estrogenic effects*

Subacute oral administration of an acetonic extract from fennel to at 0.5-2.5 mg/kg bw per day caused dose-dependent estrogenic effects: induction of the oestrus phase (after 10 days, in 40% of rats at 0.5 mg/kg, in 100% at 2.5 mg/kg), increase in mammary gland weight ($p<0.05$ at 0.5 mg/kg, $p<0.01$ at 2.5 mg/kg) and increase in weights of endometrium, cervix and vagina ($p<0.01$ to $p<0.001$ at 2.5 mg/kg). Estrogenic effects were also evident in mature male rats after treatment with the extract at 1.5 or 2.5 mg/kg bw per day for 15 days: no significant change in body or organ weights but, particularly at the higher dose, significant changes in protein and acid and alkaline phosphatase in the testes, vas deferens, seminal vesicles and prostate (Malini *et al*., 1985). An increased weight of mammal glands was also observed following administration of the fennel extract to non-ovarectomised rats (quoted by Teuscher *et al.*, 2005).

Sadrefozalayi and Farokhi (2014) evaluated the renoprotective effect of the aqueous extract of powdered fennel seeds (AEF) in experimental polycystic ovary Syndrome (PCOS) female rats. Animals were randomly divided into five groups. The first group served as control, was injected with an

equivalent volume (0.2 mL) of normal saline, and received normal diet. Animals in the second group were non PCOS rats which were treated with intragastric administration of aqueous extract of fennel (150 mg/kg bw). In the third group, the rats were treated with intraperitoneal injection of oestradiol valerate (EV) (4 mg in 0.2 mL of sesame oil). The fourth groups were treated with EV and AEF (150mg/kg bw) with the same route. The fifth groups were treated with EV and AEF (100mg/kg bw). The mean values of blood urea nitrogen in PCOS rats treated with low dose of AEF and EV and non-treated, was significantly ($p<0.05$) increased compared with non-PCOS and PCOS rats treated with high dose of AEF. Moreover, histopathological changes of kidney samples were comparable in PCOS rats with respect to treated groups with AEF.

Trans-anethole administered orally to immature female rats at 80 mg/kg bw for 3 days significantly increased uterine weight to 2 g/kg compared to 0.5 g/kg in controls and 3 g/kg in animals given oestradiol valerate subcutaneously at 0.1 µg/rat per day ($p<0.001$). The results confirmed that *trans*-anethole has estrogenic activity; other experiments showed that it has no anti-estrogenic, progestational, anti-progestational, androgenic or anti-androgenic activity (Dhar, 1995).

Estrogenic activity of *trans*-anethole at high concentrations has been determined by a sensitive and specific bioassay using recombinant yeast cells expressing the human estrogen receptor (Howes *et al*., 2002).

- *Protective effects on reproductive system*

An ethanolic extract of fennel seeds at doses of 100 and 200 mg/kg bw per day significantly increased the number of total follicles compared to the control (ethanol) group ($p< 0.05$) when administered intraperitoneally to female albino mice for five days. The numbers of graffian, antral and multilaminar follicles increased significantly in both experimental groups when compared with the control groups ($p<0.05$), however there were no significant differences in follicle numbers among the experimental groups. The number of unilaminar primary follicles did not significantly change between all groups (Khazaei *et al.*, 2011).

Azam *et al.* (2017) assessed the protective effect of an ethanolic extract of fennel seeds on mouse ovary against the destructive effects of cyclophosphamide (CP). Adult female NMARI mice were randomly divided into six groups (n=8): (A) negative control, (B) CP200 mg/kg, (C) fennel 400 mg/kg/day, (E, F, and D) that received fennel 200, 400 and 100 mg/kg per day respectively + CP200 mg/kg. The results showed that ovary weight, volume, and diameter (WVD) significantly reduced in the CP-treated groups in comparison with the A and C, but WVD increased after treatment of the mice with fennel extract, in comparison with B group ($p<0.05$). A significant decrease of serum in terms of oestrogen and progesterone levels among CP-treated groups (E, F, D and B) in comparison with the A group was observed ($p<0.05$). In the CP-treated groups a reduction in the number of different ovarian follicles in comparison with the A and C groups was observed ($p<0.05$). However, in the treated animals with fennel extract (E, F and D), these parameters significantly increased in comparison with the B group.

The protective effects of aqueous seed extracts of fennel (FVE) and caraway (CCE) on reproductive organs in female rats against cadmium chloride (CC) intoxication was investigated on a total of 36 adult female rats. Animals were divided into six groups, six in each group: control group (fed normal diet), CC-treated group (50 mg CC/kg diet), CCE-treated group (150 mg CCE/kg diet), CCE + CC group, FVE (150 mg/kg diet) and FVE + CC. One month later, all rats were sacrificed and all samples were collected at proestrus phase. Serum concentrations of estrogen, progesterone, FSH and LH were significantly decreased in CC-treated group compared to control ($p< 0.01$). The administration of FVE either alone or in combination with CC increased significantly the serum levels of both estrogen,

progesterone, FSH and LH at proestrus phase when compared to CC and control groups ($p < 0.01$) (Abdel-Wahab *et al.*, 2017).

A study was conducted to investigate the protective effects of ethanolic extracts of fennel and flaxseed seeds during pre- and post-natal period until puberty and menopause on ovarian follicular reserve (OFR). Pregnant NMRI mice received fennel (FV, 500 mg/kg/day), flaxseed (LU, 500 mg/kg/day), LU + FV (500 mg/kg/day) and no treatment was given to the controls. Female pups were studied on post-natal-days 1, 56 and 240 (PND1, 56, 240). FV and FV + LU groups showed a marked rise in body and ovary weights and diameters as compared to the control group. The number of primordial follicles at PND1, PND56, and PND240 increased significantly in the FV and FV + LU groups but decreased in the LU group compared to the control mice. There was a significant reduction in the mean of atretic follicles in the FV and FV + LU group and a marked increase in the LU group compared to the controls. Also, more apoptotic follicles were observed in the LU group, whereas less apoptotic follicles were present in the FV group. FSH and oestradiol serum levels increased significantly while LH decreased in the FV group (Pourjafari *et al.*, 2019).

- *Osteoprotective effects*

The effects of an aqueous extract of powdered fennel seeds (FvMs) on ovariectomy (OVX)-induced bone loss were evaluated using microcomputed tomography, biomechanical tests and serum marker assays for bone remodelling. Oral administration of FvMs (30 mg or 100 mg/kg/day) to female C57BL/6 mice for 6 weeks had an intermediary effect on the prevention of femoral bone mineral density (BMD), bone mineral content (BMC), and other parameters compared OVX controls. In addition, FvMs slightly decreased bone turnover markers that were accelerated by OVX (Kim *et al*., 2012).

- *Antioxidant activity of fennel extracts*

The inhibitory effects of some constituents isolated from a methanolic extract of fennel fruit were investigated on the oxidation of linoleic acid and on the activation of inactive hyaluronidase. Among the test compounds, six stilbene trimers, miyabenol C, cis-miyabenol C, foeniculoside I, foeniculoside II, foeniculoside III and foeniculoside IV, exhibited greater antioxidative activities than butylated hydroxyanisole (BHA). Furthermore, miyabenol C and cis-miyabenol C showed strong hyaluronidase inhibitory effects (Ono *et al*., 1997).

The antioxidant activities of fennel fruit and ethanolic extracts were compared *in vitro* to those of standard antioxidants such as BHA, butylated hydroxutoluene and tocopherols in various antioxidant assays including total antioxidant, free radical scavenging, superoxide anion radical scavenging, hydrogen peroxide scavenging, metal chelating activities and reducing power. The aqueous extract was prepared by stirring 25 g of powdered herbal substance in 500 mL boiling water for 15 min., then filtering and lyophilizing; the ethanolic extract was prepared by extracting 5 times 25 g of herbal substance with 100 mL ethanol until the extraction solvents became colourless (total solvent volume is 500 mL), then the extract was filtered and dried. The extracts were re-dissolved in distilled water to test the antioxidant activity. Both fennel extracts showed effective reducing power, free radical scavenging, hydrogen peroxide scavenging and metal chelating activities (Oktay *et al*., 2003).

Antioxidant activity of an aqueous extract from fennel fruit (as evaluated in vitro from amounts needed for 50% scavenging of superoxide radicals or for 50% inhibition of lipid peroxide or for 50% inhibition of hydroxyl radicals) was found to be higher than that of ascorbic acid and comparable to those of other umbelliferous fruits (*Cuminum cyminum, Anethum graveolens*). The aqueous extract was prepared from 1 kg of dried powdered fruit extracted with boiling water for 30 min., filtered,

evaporated until the yield of 100 g and redissolved in distilled water for assessment of antioxidant activity (Satyanarayana *et al*., 2004).

The hot water extracts of 13 spices commonly used in meat processing plants, including fennel, were examined for their anti-oxidant activity. The DPPH radical scavenging ability of the fennel extract (10.48%) was much lower than that of ascorbic acid ($p<0.05$). The superoxide radical scavenging activity of hot water extracts of fennel was slightly higher compared to ascorbic acid (36.48%) at the same concentration of 0.5 mg/mL. Finally, the hydroxyl radical scavenging activities of fennel extract and ascorbic acid were close (44.63& vs 48.72%) (Kim *et al.*, 2011).

The antioxidant activity of a methanolic extract of dried, powdered fennel seeds was determined by DPPH free radical scavenging, metal induced protein and lipid oxidation inhibition and protection of DNA against H2O2 induced damage. Fennel exhibited a concentration-dependent free radical scavenging activity with IC50 of 2.1 mg dry seed weight. IC_{50} observed for protection of protein and lipids against copper ion induced oxidation was 2.1 and 2.5 mg, respectively. Extract equivalent to 0.5 µg seeds was able to protect DNA against H_2O_2 induced oxidation (Dua *et al.*, 2013).

Antioxidant potentials of methanol extracts of cumin and fennel were determined using free radicals scavenging activity, reducing activity and lipid peroxidation inhibition potential. The extracts have been prepared by adding 50 mL of 80% methanol to 20 g of powdered cumin or fennel at room temperature for 24 h; the obtained extract was then filtered, centrifugated and concentrated. Methanol extracts from cumin revealed greater free radical scavenging activities, reducing activity and peroxidation inhibition potential compared to fennel ($p<0.05$). These activities were dose-dependent and increased with increasing concentration (Rani & Meena, 2014).

- *Hepatoprotective effect*

A methanolic extract prepared from dried, grounded fennel seeds (FSE) and a whey protein concentrate (WPC) were administered orally to adult male Sprague-Dawley rats with tienilic acid (TA)-induced hepatotoxicity for six weeks at doses of 200 mg/kg bw daily and 0.5 g/kg bw per day, respectively. The administration of either WPC or FSE to TA-treated animals significantly protected the liver against the injurious effects of tienilic acid. This appeared from the improvement of hepatic functions, atherogenic markers, Na+/K+ATPase activity, endogenous antioxidants and hepatic lipid peroxidation level; where WPC showed the strongest protection effect (Abdel-Wahhab *et al.*, 2016).

- *Hypotensive effect*

A lyophilised aqueous extract (obtained by boiling 10g of fennel in 100 mL of water for 10 min.) administered orally at 190 mg/kg bw (equivalent to crude herbal substance at 1000 mg/kg) for 5 days significantly lowered the systolic blood pressure of spontaneously hypertensive (SH) rats ($p<0.05$), but had no effect on normotensive rats. The extract also significantly increased the urine output of SH rats, by 80% at day 3 ($p<0.05$), and increased renal excretion of sodium and potassium ($p<0.05$), suggesting that fennel acts mainly as a diuretic and natriuretic in the SH rats (El Bardai *et al.*, 2001).

- *Hypoglycemic effect*

Ten ethanol extracts from Indonesian plants, including *Foeniculum vulgare* (seeds) were selected for *in vitro* inhibitory activity of dipeptidyl peptidase - IV (DPP-IV). Fennel extract at a concentration of 1000 ppm resulted in a percentage of inhibition of 46.15%, whereas sitagliptin (positive control) showed 85.18% of inhibition (Setyaningsih *et al.*, 2019).

Ethyl acetate, *n*-butanol and benzene extracts of *Foeniculum vulgare* Mill. Seeds were screened for their *in-vitro* antidiabetic activity by α-amylase and α-glucosidase inhibition assay. The obtained results

showed that all the three extracts had high alpha-glucosidase inhibitory affect than the alpha-amylase inhibition when compared with the standard acarbose values (Godavari *et al.*, 2018).

The aqueous extracts of *Cassia angustifolia* (EACA) leaves and *Foeniculum vulgare* (EAFV) seeds were assessed for their potential anti-diabetic effects on albino rats with diabetes induced by an intraperitoneal injection of a single dose of STZ. Diabetic rats received either EACA (150 mg/kg/day), or EAFV (150 mg/kg/day) and their combination by gastric intubation for 30 days. Daily oral ingestion of EACA and/or EAFV extract for 4weeks after diabetes induction ameliorated hyperglycemia, increased insulin ($p<0.01$), improved lipid profiles, restored body weight loss and liver function, blunted the increased in MDA, modulated the levels of hepatic and pancreatic SOD and CAT activities and GSH content ($p<0.01$), compared to the untreated diabetic group (Osman *et al.*, 2017).

- *Antiurolithiatic effect*

The antiurolithiatic property of an ethanolic extract of powdered seed of *Foeniculum vulgare* was evaluated using ethylene glycol as a urolithiatic agent in male Wistar rats. Prophylactic and curative urolithiasis models were used with 5 groups of 6 rats in each model. Ethanolic extract of fennel seeds in three doses 100, 200, and 300 mg/kg was used. Cystone 750 mg/kg was used as a standard drug. All drugs were administered orally. Zinc discs were surgically implanted in the bladder in all rats. After recovery, rats in the prophylactic model received three different doses of ethanolic extract of fennel seeds along with 1% ethylene glycol for 2 weeks whereas the rats in the other model received 1% ethylene glycol for 2 weeks followed by an ethanolic extract of fennel seeds in three doses for the next 2 weeks. In both the models, all three doses of an extract of fennel seeds were effective in reducing stone formation as compared to control group with $p<0.05$. In both the models, all three test doses were comparable with cystone, but 300 mg/kg extract in prophylactic showed significance ($p<0.05$) when compared to standard (Poojar *et al.*, 2017).

- *Nephroprotective effect*

Alcoholic extract of fennel seeds played a protective role against the renal toxicity induced by Cisplatin. Male rabbits were randomly divided to three equal groups (10 animals/group). The first group (G1) administered 1 mL/kg normal saline and was considered as a control group. The second group (G2) was injected with Cisplatin (Cis) solution intra-peritoneally (IP), once at a dose of 6mg/kg bw for induction of renal failure, while the third group (G3) was injected IP with 6 mg/kg of Cis + oral dosage of 60 mg/kg of fennel extract for three months. The injection of Cis resulted in a significant increase ($p<0.05$) in the concentration of Cr, uric acid, Ur, K+, MDA, peroxy nitrite and TNF-α. Comparative to control group and significant decrease ($p<0.05$) in Na+ and GSH comparative with control group. The group treated with the alcohol extract of the fennel plant showed significant decrease ($p<0.05$) in the concentration of Cr, uric acid, Ur, K+, MDA and TNF-a comparative with G2 and no significant differences in Na+ and GSH concentration comparative with control group (Abd-Alsalam Alsalame et al., 2018).

- *Cytotoxic effect*

The effect of aqueous extract of sweet fennel alone and in combination with 50 µg/mL cisplatin was evaluated on a human cervical cancer adenocarcinoma cell line (HeLa cells). Increasing the concentration of sweet fennel from 50-80 µg/mL decreased the percentage of cell viability of HeLa cells from 86.74% to 78.28%, respectively. Further decrease to 11.31% was demonstrated when 50 µg/mL of fennel was combined with 50 µg/mL cisplatin (additive effect). In addition to the signs of apoptosis observed in HeLa cells at 50 µg/mL of fennel, disruption of both nuclear and cytoplasmic membranes and presence of auto phagolysosomes were noticed at a dose of 80 µg/mL. Combining 50 µg/mL of

cisplatin with 60, 70, and 80 µg/mL of sweet fennel revealed no significant difference in comparison to cisplatin alone. The combination with 50 µg/mL of sweet fennel revealed marked vacuolization of the cytoplasm, fragmentation of the nucleus, and complete disruption of the nuclear membrane (Sait, 2015, only abstract available).

An aqueous extract of fennel (25, 50, 75 and 100 µg/mL) decreased the cell viability of breast carcinoma cells in a concentration-dependent manner and the IC_{50} against MCF-7 cell line was 73.9 µg/mL (Bano *et al.*, 2019).

- *DNA-protecting effects*

Jayakumar and Kanthimathi (2012) analysed the DNA protecting activity and inhibition of nicotine-induced cancer cell migration of 9 spices, including fennel. Murine fibroblasts (3T3-L1) and MCF-7 cells were pre-treated with an ethanolic extract of fennel and then exposed to H2O2 (25, 50 and 100 µM) and nicotine (10 µM). The comet assay was used to analyse the DNA damage. Fennel at concentrations of 25 and 50 µg/mL significantly showed a significant decrease ($p<0.05$) in comet tail length compared to the control of H2O2 treatment alone. Treatment of MCF-7 cells with nicotine induced cell migration, whereas pre-treatment with fennel 25, 50 and 75 µg/mL reduced this migration dose-dependently ($p<0.05$ compared to cells treated with nicotine alone).

- *Other effects*

Anethole was reported to have a contractile effect on smooth muscle (Reiter and Brandt, 1985).

It was also reported to increase the pentobarbital-induced sleeping time in mice (Marcus and Lichtenstein, 1982).

3.1.3. Safety pharmacology

No data available.

3.1.4. Pharmacodynamic interactions

No data available.

3.1.5. Conclusions

Powdered fennel fruits, water and alcoholic extracts of fennel fruits showed a number of effects on gastrointestinal tract in pre-clinical *in vivo* and *in vitro* studies. These include a spasmolytic action on smooth muscles of stomach, ileum and jejunum and an increase of gastric acid secretion.

Fennel fruit extracts exhibited *in vitro* strong inhibitory activities against the growth of a wide spectrum of bacteria and fungi known to be pathogenic for man and other species. These anti-microbial effects might concur in relieving bloating and flatulence.

An infusion of bitter fennel seeds increased mucociliary transport velocity *in vitro*; fenchone augmented volume output of respiratory tract fluid whereas fenchone and anethole decreased its specific gravity in *in vivo* experiments on rabbits.

Alcoholic extract of fennel seeds and *trans*-anethole have anti-inflammatory effects in several *in vivo* and *in vitro* studies acting on different inflammatory mediators such as by inhibiting 5-LPO, decreasing the level of LDH, IL-6 and TNF-α or reducing IL-6 and IL-10 mRNA expression.

All these effects are coherent with the long-standing use of fennel as spasmolityc in mild, spasmodic gastro-intestinal complaints and as expectorant in cough associated with cold, although in most of pre-clinical studies the variety (*dulce* or *vulgare*) of fennel is not reported.

3.2. Overview of available pharmacokinetic data regarding the herbal substance(s), herbal preparation(s) and relevant constituents thereof

No data are available on absorption, metabolism and excretion of fennel in human beings or animals.

After oral administration of radioactively-labelled *trans*-anethole (as the methoxy-^{14}C compound) to 5 healthy volunteers at dose levels of 1, 50 and 250 mg on separate occasions, it was rapidly absorbed. 54-69% of the dose (detected as ^{14}C) was eliminated in the urine and 13-17% in exhaled carbon dioxide; none was detected in the faeces. The bulk of elimination occurred within 8 hours and, irrespective of the dose level, the principal metabolite (more than 90% of urinary-^{14}C) was 4-methoxyhippuric acid (Caldwell and Sutton, 1988). *Trans*-anethole is metabolised in part to the inactive metabolite 4-methoxybenzoic acid (Schulz *et al*., 1998). An earlier study with two healthy subjects taking 1 mg of *trans*-anethole gave similar results (Sangster *et al*., 1987).

In mice and rats *trans*-anethole is reported to be metabolised by O-demethylation and by oxidative transformation of the C3-side chain. After low doses (0.05 and 5 mg/kg bw) O-demethylation occurred predominantly, whereas higher doses (up to 1500 mg/kg bw) gave rise to higher yields of oxygenated metabolites (Sangster *et al*., 1984a and 1984b).

Experimental studies on rats and mice showed that anethole is completely absorbed but slowly after oral administration. The major metabolic pathways involve O-demethylation, oxidation of the C3-side chain, and conjugation with glucuronic acid, glycine, sulfate, and glutathione. The main metabolites of anethole are 4-methoxy-hippuric acid, 4-methoxy-benzoic acid, 4-hydroxypropenylbenzene, 2-hydroxy-1-methylthio-1-(4'-methoxyphenyl)-propane, 4-methoxy derivatives of acetophenone, cinnamic alcohol, and cinnamic acid. The elimination of anethole occurs within 48–72 h, and the major routes are renal, pulmonary, and faecal excretion. The metabolism and excretion of anethole are dose-dependent in animals. Low doses of anethole are mainly metabolised via O-demethylation and eliminated via exhalation as CO2. With increasing doses, the metabolism of anethole involves side-chain oxidation and epoxidation, and the renal excretion predominates (Aprotosoaie *et al.*, 2016).

The metabolism of estragole was thoroughly studied in order to clarify the source of carcinogenicity. Four common main metabolites detected in the urine of rats and mice treated with estragole-^{14}C were 1'-hydroxyestragole, 4-methoxycinnamyl alcohol, 4-methoxyphenyllactic acid, and 4-methoxyhippuric acid. In rats, most of the radioactivity (mean 54%) was exhaled as CO2-^{14}C, resulting from demethylation of the estragole-^{14}C with the radioactive label at the methoxy group. Thus, p-allylphenol was probably one of the most abundant intermediate metabolites. Among multiple pathways resulting in reactive metabolites, the hydroxylation at the 1'-carbon to 1'-hydroxyestragole and subsequent sulfotransferase (SULT)-catalysed conversion to 1'-sulfoxyestragole was the most important step. Recently, N-acetyl-S-[3'-(4-methoxyphenyl)allyl]-L-Cys (AMPAC) has been observed in human urine samples after consumption of fennel tea. The formation of this further metabolite has been supposed to derive by the detoxification of 1'-sulfoxyestragole by glutathione (Monien *et al.*, 2019).

Investigations showing liver enzymes-inducing effects of compounds present in fennel oil strongly raise the possibility for interactions of fennel with other medicinal products to take place. Fennel was tested for its *in vitro* CYP1A2, 2D6, and 3A4 inhibitory potential. An aqueous extracts of fennel was made from commercially available herbal products, and incubations were performed with recombinant cDNA-expressed human CYP enzymes in the presence of positive inhibitory controls. Metabolite formation

was determined by validated LCMS/MS or HPLC methodologies. IC_{50} inhibition constants were estimated from CYP activity inhibition plots using non-linear regression. Inhibition was shown for fennel towards CYP2D6 and 3A4 with respective IC_{50} constants of 23 ± 2 and 40 ± 4 µg/mL. Based on the recommended dosing of the commercial herbal products investigated, clinically relevant systemic CYP inhibitions could be possible for fennel. In addition, fennel might cause a clinically relevant inhibition of intestinal CYP3A4 (Langhammer & Nilsen, 2013). However, this *in-vitro* findings does not justify a specific mention in the monograph.

3.3. Overview of available toxicological data regarding the herbal substance(s)/herbal preparation(s) and constituents thereof

3.3.1. Single dose toxicity

Oral administration of an ethanolic extract of fennel to mice at 0.5, 1 and 3 g/kg bw caused no mortality and no significant difference in body and vital organ weights or in external morphological, haematological or spermatogenic parameters in comparison with the control group over a period of 24 hours (Shah *et al*., 1991).

Fennel extracts in high dosages resulted in abnormal movements, tremor, fasciculation and drowsiness in experimental animals (Ostad *et al*., 2000).

Oral LD_{50} values per kg bw were determined for *trans*-anethole as 1.8-5 g in mice, 2.1-3.2 g in rats, and 2.16 g in guinea pigs; intraperitoneal LD_{50} values for *trans*-anethole were determined as 0.65-1.41 g/kg bw in mice and 0.9-2.67 g/kg bw in rats (Lin, 1991).

Anethole activates the central nervous system and its excessive use may lead to convulsions (Zargari, 1992).

3.3.2. Repeat dose toxicity

Oral administration in drinking water of an ethanolic extract of fennel (grounded fruits were extracted with 95% ethanol and the solvent evaporated to semi-solid residue) to mice at 100 mg/kg bw per day for three months caused significant body weight gain in male mice (n=10) and weight loss (n=10) in females. Alopecia was observed in 3/10 males, swollen testes in 1/10 males and penile erection in 2/10 males. No toxic symptoms were observed in females. It caused no significant differences in mortality or in haematological and spermatogenic parameters in comparison with the control group (Shah *et al.*, 1991).

Male rats receiving 0.25% of anethole in their diet for one year showed no toxic effects, while other receiving 1% for 15 weeks had slight oedematous changes in liver cells (Hagan *et al*., 1967).

In 90-day experiments in rats, 0.1% of *trans*-anethole in their diet caused no toxic effects. However, dose-related oedema of the liver was reported at higher levels of 0.3%, 1% and 3%, which have no therapeutic value (Lin, 1991).

Rats given *trans*-anethole as 0.2, 0.5, 1 or 2% of their diet for 12-22 months showed no effects at any level on clinical chemistry, haematology, histopathology or mortality. Slower weight gain and reduced fat storage were noted at the 1% and 2% levels (Lin, 1991).

3.3.3. Genotoxicity

- *Mutagenicity*

Aqueous and methanolic extracts of fennel (50 g finely powdered extracted with 300 mL of water or methanol at 40°C for five hours with manual shaking at intervals and then dried) did not show any mutagenic activity in the Ames test using *Salmonella typhimurium* strains TA 98 and TA 100, with or without S9-metabolic activation (Morimoto *et al*., 1982; Yamamoto *et al*., 1982), whereas fennel oil (2.5 mg/plate) was mutagenic (Marcus and Lichtenstein, 1982). The two extracts showed no activity also in the *Bacillus subtilis* rec-'assay (Morimoto *et al*., 1982).

The genotoxic potential of methanolic extracts of plant food supplements (PFS) derived from various botanicals, such as basil, parsley, fennel, nutmeg and sassafras were tested by using both alkaline Comet assay (strand breaks, alkali labile sites, and cross linking evaluation) and micronucleus test (clastogenic and aneugenic damage) after 24h of treatment in HepG2 cells (derived from human hepatoma). At the tested concentrations (20-800 µg/mL) the extracts of PFS did not show either cytotoxic or genotoxic activity, even when they contain appreciable amount of alkenylbenzenes. Moreover, the extracts inhibited dose-dependently the DNA damage induced by the 1'-hydroxymetabolites, when tested together (Marabini *et al.*, 2014, only poster available).

The genetic damage and cytostatic effects of fennel powdered seeds (FSPw) and fennel seeds essential oil (FSEO) was assessed in HepG2 cells. According to the data obtained from the comet assay, after 4h exposure, none of the tested concentrations of FSPw (10, 20 and 40 µg/mL) and FSEO (0.5, 1.0 and 2.0 µl/mL) induced a significant increase in percentage of DNA in the comet tail (*i.e.,* tail intensity, %), as compared to control (untreated) cells. The induction of chromosomal aberrations was examined using the CBMN test; after 24 h of exposure to the tested compounds, the frequencies of MN in treated cells did not vary significantly from those of the respective controls, whereas positive control cells showed significantly increased micronucleated cell frequencies (MN ‰=11.67 ± 1.09; $p< 0.05$). In addition, FSPw failed to induce apoptosis and cell cycle perturbation, confirming the absence of DNA damage, whereas FSEO showed a dose-dependent increase in the proportion of early (p=0.001) and late (p=0.001) apoptotic cells. FSEO caused a dose-dependent decrease of the proportion of G0/G1 cells (p=0.001) with a concurrent accumulation of cells at G2/M phase (p=0.002) (Levorato *et al.*, 2018).

- *Anethole*

Tested in *Salmonella* mutagenesis assay and also in mouse lymphoma L5178Y $TK^{+/-}$cell mutagenesis assay, anethole was inactive in *Salmonella thyphimurium* tester strains TA1535, TA1537, TA15358, TA98 and TA 100 and was active in the mouse lymphoma assay only with Aroclor 1254-induced rat liver S9 activation (Heck *et al.*, 1989).

In the *Salmonella*/microsome mutagenicity assay with Aroclor 1254-induced rat liver S9 activation performed with *Salmonella thyphimurium* tester strains TA1535, TA1537, TA15358, TA98 and TA 100 showed that anethole may have a very weak activity for strain TA100; however, no obvious dose-related response can be found (Hsia *et al.*, 1979).

Mortelmans *et al.* (1986) reported negative results of mutagenicity testing of anethole performed in the *Salmonella* pre-incubation assay, which is a modification of the standard plate incorporation assay, using four *Salmonella* strains (TA1535, TA1537, TA98 and TA100) in the presence and absence of rat and hamster Aroclor 1254-induced liver S9 activation.

The mutagenic activities of anethole and its metabolite 3'-hydroxyanethole were studied using three tester strains of *Salmonella thyphimurium (*TA1535, TA00, TA98). Addition of an NADPH-generating system and liver S13 fraction from Aroclor-treated rats (6.8 mg liver/protein plate) to the incubation mixture of TA100 tester strain increased mutagenic activities. Approximately 45 revertants were

obtained per µmol of anethole. Under the same conditions, 3'-hydroxyanethole showed no significant mutagenic activity with less than 7 µmol/plate. Above this concentration the S13-mediated mutagenicity increased linearly with increased doses up to 15 µmol/plate (about 1000 revertants with 15 µmol/plate) (Swanson *et al.*, 1979).

Five strains of *Salmonella thyphimurium* (TA1535, TA1537, TA1538, TA98 and TA100) with and without S9 fractions from Aroclor 1254-induced rats were used to study potential mutagenic effects of *trans*-anethole. The lowest overtly toxic concentration for trans-anethole was 1 mg/plate. No mutagenic activity was observed at concentration of up to 50 µg trans-anethole/plate with or without metabolic activation. However, the addition of 3'phosphoadenosine-5'phosphosulphate (PAPS) to the microsomal assay markedly increases the mutagenicity of trans-anethole in TA1535 tester stain. The mutation rate observed was approximately 4, 5, 10, 11, 9 and 3 times that of the background rate at *trans*—anethole concentrations off 0.05, 0.20, 1.0, 5.0, 15.0 and 50.0 µg/plate respectively (To *et al.*, 1982).

Gorelick in 1995 reviewed nine previously conducted gene mutation studies (Heck *et al.*, 1989; Hsia *et al.*, 1979; Marcus & Liechtenstein, 1982; Mortelmans *et al.* 1986; Nestmann *et al.*, 1980; Sekizawa & Shibamoto, 1982; Swanson *et al.*, 1979; To *et al.*, 1982) and repeated the *Salmonella* /microsome test as well as the L5178Y mouse lymphoma TK +/-assay to ascertain their reproducibility and relevance. In the nine studies reviewed, anethole was uniformly negative in the Salmonella tests to detect base-pair substitutions or frameshift mutations without metabolic activation and this was also the case in four studies with metabolic activation after careful consideration of all experimental conditions. The studies which suggested a weak mutagenic potential of anethole (Marcus & Liechtenstein, 1982; Swanson *et al.*, 1979; Mortelmans *et al.*, 1986; Sekizawa & Shibamoto, 1982) were the result of the use of non-standard protocols (using longer pre-incubation times, excessive quantities of S-9 protein and/or the addition of co-factors) and have also been found to be irreproducible (Gorelick, 1995).

Gorelick (1995) reported dose-dependent response of *trans*-anethole only in the mouse lymphoma assay with metabolic activation. Anethole was found to be mutagenic in the mouse lymphoma assay, which is known for its extreme sensitivity and poor selectivity for genotoxicity also by other authors (Heck *et al.*, 1989; Caldwell, 1993).

Other results showing the absence of mutagenic potential of anethole include assays in *Escherichia coli* (Sekizawa & Shibamoto, 1982) and in *Saccharomyces cerevisiae* (Nestmann & Lee, 1983).

A mouse micronucleus assay was negative, with no micronuclei found at 6 and 30 hours after anethole i**ntraperitoneal** administration to groups of 5 male and 5 female mice in two doses of 0.25 or 0.5 g/kg bw (Marzin, 1979; in Lin, 1991). Similarly no significant increase in genotoxicity was observed in the mouse bone marrow micronucleus test after the oral pre-treatment of mice with trans-anethole at 40-400 mg/kg bw 2 and 20 hours before intraperitoneal injection of genotoxins; a moderate, dose-dependent protective effects against known genotoxins such as cyclophosphamide, pro-carbazine, N-methyl-N'-nitrosoguanidine, urethane and ethyl methane sulfonate was observed ($p<0.05$ to $p<0.01$ at various dose levels) (Abraham, 2001).

Very low levels of DNA adduct (<1.4 pmol/mg DNA) were observed after administration of anethole to mice, whereas 150 and 220 times as many adducts were detected following administration of safrole and estragole, respectively (Phillips et al*.,* 1984).

Unscheduled DNA synthesis (UDS) assays in rat hepatocytes did not indicate any mutagenic potential of anethole (Howes *et al.*, 1990; Muller *et al.*, 1994).

Anethole has three primary metabolites in the rat and the pathway of toxicological concern is that of epoxidation of the 1,2 double bond at the side chain; in fact, 3'-hydroxylation does not result in genotoxicity or marked cytotoxicity and O-demethylation is a detoxication reaction (Sangster *et al.*, 1984a and 1984b; Bounds & Caldwell, 1996). Cytotoxicity of anethole is enhanced when the cellular epoxide defence mechanisms of conjugation with reduced glutathione and hydration by cytosolic epoxide hydrolase are severely compromised. However, modulation of epoxide metabolism by the same mechanism in cultured cells failed to induce UDS by anethole nor was there a UDS response in hepatocytes of female rats dosed with anethole *in vivo* (Marshall & Caldwell, 1996). The synthetic epoxide of anethole was also tested and found to be cytotoxic, but not genotoxic. The lack of induction of UDS by anethole epoxide provided a further support to the hypothesis that marginal hepatocarcinogenesis observed in female rats given 1% anethole in the diet for 121 weeks was not initiated by a genotoxic event (Marshall & Caldwell, 1996).

In the 51st meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) a document on safety evaluation of *trans*-anethole was prepared; the conclusions were that *trans*-anethole and its metabolites are unlikely to be genotoxic *in vivo*; the cytotoxic metabolite, anethole epoxide, was suggested to be the possible causative agent of the hepatotoxic effect observed in pre-clinical studies in rats. The report of JECFA allocated the acceptable daily intake (ADI) at the dose of 0-2 mg/kg bw on the basis of scientific pre-clinical data published on *trans-anethole* (JECFA, 1999).

In 1999 the USA Expert Panel of FEMA (Flavour and Extract Manufacturers' Association) released a review of scientific data relevant to the safety evaluation of *trans-anethole* as a flavouring substance. The review concluded that trans-anethole does not represent a carcinogenic risk to humans and can be "generally recognised as safe" (GRAS) at low level of intake (54 μg/kg bw per day) (Newberne *et al.*, 1999).

- *Estragole*

The genotoxicity of estragole has been assessed in the "Public Statement on the use of herbal medicinal products containing estragole" (EMA/HMPC/137212/2005 Rev 1 Corr 1*).

3.3.4. Carcinogenicity

FSME (Fennel Seed Methanolic Extracts), 100 mg/kg, intraperitoneal, reduces oxidative stress and it protects the EAC (Ehrlich Ascites Carcinoma) cells of Swiss albino mice from the damages caused by reactive oxygen species. (Mohamad *et al.*, 2011).

An aqueous extract prepared from powdered fennel seeds was assessed for its anti-tumour and anti-metastatic effects on adult female BALB/C mice with breast cancer induced by subcutaneous injection of 4T1 cells in the right lower flank. The mice received fennel extracts daily via intraperitoneal injection for two weeks. Tumour size significantly decreased after nine days treatment of the fennel 100 and 200 mg/kg. Fennel treatment 100 and 200 mg/kg caused an increase in the ratio of the expression of E-cadherin to Ki-67 and dysadherin in the tumour tissues. On the other hand, the expression of Ki-67 and HER2 decreased in the ovary (Mehralikhani *et al.*, 2020).

- *Anethole*

From a series of studies investigating the effect of anethole when added to female CD-1 mice diet or given orally or by intraperitoneal injection to male pre-weaning B6C3F1 mice, Miller et al. (1983) concluded that anethole was not a hepato-carcinogen; although these studies were not carried out for test animal lifetimes. Safrole and estragole were found to be highly active as liver carcinogens in both

these tests.

In another bioassay carried out in Sprague–Dawley (SD) rats, 0.25, 0.5, or 1.0% anethole was administered in the diet for 121 weeks. Results showed the occurrence of a small, but statistically significant, incidence of hepatocellular carcinomas in female rats receiving 1% anethole (Truhaut *et al.*, 1989). These hepatocellular carcinomas were associated with other changes to the liver (increase in relative liver weight) similar to those observed after enzyme induction (Newberne *et al.*, 1989) and were considered not to be caused by a direct genotoxic effect of *trans-anethole* (Lin, 1991). Reed & Caldwell (1992) also showed that intraperitoneal administration of anethole to SD rats increased liver weight, microsomal protein and cytochrome P450 (CYP450) content.

In Swiss albino mice with Ehrlich ascites tumour (EAT) in the paw, anethole administered orally at 500 or 1,000 mg/kg on alternate days for 60 days significantly and dose-dependently reduced tumour weight ($p<0.05$ at 500 mg/kg, $p<0.01$ at 1,000 mg/kg), tumour volume ($p<0.01$ at 500 mg/kg, $p<0.001$ at 1,000 mg/kg) and body weight ($p<0.05$ to 0.01) compared to EAT-bearing controls. Mean survival time increased from 54.6 days to 62.2 days (500 mg/kg) and 71.2 days (1,000 mg/kg). Histopathological changes were comparable to those after treatment with cyclophosphamide (a standard cytotoxic drug). These and other results demonstrated the anti-carcinogenic, cytotoxic and non-clastogenic nature of anethole (Al-Harbi *et al*., 1995).

Anethole at a concentration below 1 mM has been shown to be *in vitro* a potent inhibitor of tumour necrosis factor (TNF)-induced cellular responses, such as activation of nuclear factor-kappa B (NF-κB) and other transcription factors, and also to block TNF-induced activation of the apoptotic pathway. This might explain the role of anethole in suppression of inflammation and carcinogenesis (Chainy *et al*., 2000).

- *Estragole*

The carcinogenicity of estragole has been assessed in the "Public Statement on the use of herbal medicinal products containing estragole" (EMA/HMPC/137212/2005 Rev 1 Corr 1*).

For estragole, metabolic activation pathway and DNA adduct formation are amply demonstrated in animals (mice and rats) and the same pathway is operative in human *in vitro* systems. There is general consensus that adduct formation is causally related to tumorigenesis, unless there are specific and biologically persuasive reasons to the contrary. In a view of HMPC the mode of action for tumour formation is relevant for humans (EMA/HMPC/137212/2005 Rev 1 Corr 1*).

The major metabolic pathways of estragole have been well characterised in rats and mice *in vitro* and *in vivo* and studies have been published on *in vitro* metabolism of estragole in human hepatic preparations. Three major metabolic pathways have been established:

1) O-demethylation resulting 4-allylphenol and more distal metabolites (identified as a detoxification pathway);

2) 1'-hydroxylation, which is a proximal active metabolite undergoing sulfoconjugation to 1'-sulfooxyestragole capable of binding to DNA and protein;

3) epoxidation of the allyl side chain leading to estragole-2',3'-epoxide, which is rapidly metabolised 145 by epoxide hydrolase and glutathione transferase to detoxified metabolites (also identified as a detoxification pathway).

Although toxicokinetics and metabolism of estragole have not been thoroughly studied in humans, there is evidence that under *in vivo* administration of estragole to humans, the liver is exposed to the

compound and the first step in metabolic activation, the formation of 1'-hydroxyestragole, is possible. In the view of Punt (2009), in spite of significant differences in the relative extent of different metabolic pathways between human and male rat it is probable that there is a minor difference on the ultimate overall bioactivation of estragole to 1'-sulfooxyestragole. As the processess observed *in vitro* on humans liver microsomes, have some similarities to those in rodents (Jeurissen, 2007) in which carcinogenicity has been observed, the occurrence of the same mechanism can be regarded as possible (EMA/HMPC/137212/2005 Rev 1). Recently, the hypothesis that in humans 1'-sulfooxyestragole may be detoxified by glutathione conjugation has been confirmed on 12 volunteers taking 500 mL of fennel tea containing 2.2 mg of estragole was supported by the finding of N-acetyl-S-[3′ -(4-methoxyphenyl)allyl]-L-Cys (AMPAC), a mercapturic acid, in urine samples (Monien *et al.*, 2019).

3.3.5. Reproductive and developmental toxicity

Malini *et al*. (1985) showed estrogenic activity of an acetone extract of *Foeniculum vulgare* seeds in both male and female rats (the powdered material was extracted with acetone with Soxhelet, then dried and dissolved with a known volume 1% ethanol for oral administration; no further information). The protein concentration was found to be significantly decreased in testes and *vas deferens* and increased in seminal vesicles and prostate gland, following oral administration of the extract at the doses of 1.5 mg/kg and 2.5 mg/kg for 15 days in male rats. Moreover, the activity of acid and alkaline phosphatase decreased in all these tissues. Only the alkaline phosphatase was unchanged in deferent duct. The same doses of the extract, after oral administration for ten days in female rats, caused vaginal cornification and estrus cycle. The lower doses caused increase in weight of mammary glands and the higher doses increased the weight of oviduct, endometrium, myometrium, cervix and vagina.

The potential anti-fertility effects of dry ethanolic extract (prepared by drying of an extract obtained by maceration of powdered fennel seed with 70% ethanol for 48 h) was studied on male rats. Forty Wistar rats were divided into five equal groups. The control group received distilled water and the experimental groups were orally administered 1 mL of hydro-alcoholic extract of fennel seed in four doses of 35, 70, 140, and 280 mg/kg bw daily for 60 days. After the last gavage, the rats were anaesthetised and the caudal part of the right epididymis was used for sperm counting. After fixation of the testes, microscopic sections were prepared and histological changes were evaluated. The number of spermatogonia after doses of 140 and 280 mg/kg and Sertoli cells after a dose of 140 mg/kg decreased significantly as compared with the control group ($p < 0.05$). The number of primary spermatocytes and sperm count decreased significantly in the experimental groups (70, 140, and 280 mg/kg) when compared to the control group ($p < 0.05$). Furthermore, thickening of the basement membrane, cell apoptosis, and irregular arrangement of the germinal epithelium were observed in the experimental groups (Mansouri *et al.*, 2016).

In another experimental study, 48 female BALB/c pups were divided into four groups: control (without treatment), treatment with oestradiol benzoate (EB), 100 and 200 (mg/kg bw) fennel dry ethanolic extract FEE that were daily administered for five days from birth. Powdered dried seeds (100 g) were added to 1000 mL of 70% ethanol (V/V) and left to soak at 25ºC for 20 h; the extract was filtered and dried at low pressure at 30°C. The age at vaginal opening (VO) was significantly earlier in EB and 200 FEE. Body weight at VO was lower than control only in EB. A disrupted oestrus cycle decreased number of cycles and increased index diestrus were observed in EB and 200 FEE treated mice. Ovary weight in the EB-, 100- and 200 FEE-treated groups were lower. The number of Graafian follicles in the EB-, 100- and 200 FEE and number of corpus luteum in the EB and 200 FEE groups were lower than that of control females. Oestradiol concentration increased in the EB and 200 FEE and LH concentration

decreased in the EB-, 100- and 200 FEE groups. The lordosis quotient (LQ) was significantly low in the EB- and 200 FEE-treated groups, vis-à-vis the control group (Parandin & Yousofvand, 2019).

Shayan *et al.* (2019) evaluated the effect of a concentrated aqueous extract of *Foeniculum vulgare* on fetal development in pregnant mice models. A dry extract was prepared by dissolving 40 g of milled powder of *Foeniculum vulgare* seeds in 200 mL distilled water, refluxing the mixture for 2 h; then the extract was filtered and dried. A total number of 24 female BALB/c mice with a weight range of 25-30 g was divided into four groups. Each group received 0.25 mL of the extract with different concentrations (2.5, 12.5, and 25 mg) and distilled water as a control group. The aqueous extract of fennel was administered through oral gavage on a daily basis from day 6 to day 15 of pregnancy. On day 16, the fetuses were analysed in terms of morphological changes, skeletal disorders, and cellular alterations. The result showed the dose-dependent teratogenic effect of the aqueous extract of *Foeniculum vulgare*. At 12.5 and 25 mg concentration, the teratogenic effect was more severe. Oral gavage administration of the aqueous extract of *Foeniculum vulgare* increased the number of dead fetuses and reduced the average weight, height, and crown-rump length. Subcutaneous hemorrhage, dorsal lesion, wrinkled skin, and considerably lower than normal fetal weight observed in gross morphological inspection at 25 mg concentration. The skeletal studies revealed fetal anomalies, reduction in ossification and reduction in the number of ribs. Internal bleeding around the liver and lungs, pulmonary fibrosis and disruption in the arrangement of hepatocytes was also observed in histological analysis.

Trans-anethole exerted a dose-dependent, anti-implantation activity after oral administration to adult female rats on days 1-10 of pregnancy. When compared with control animals (all of which delivered normal offspring on completion of term), *trans*-anethole administered at 50, 70 and 80 mg/kg bw inhibited implantation by 33%, 66% and 100% respectively. Further experiments were conducted with the 80 mg/kg dose at different stages of pregnancy. When rats were administered *trans*-anethole on days 1-2 of pregnancy, normal implantation and delivery occurred. However, rats administered anethole on days 3-5 of pregnancy, implantation was completely inhibited; and in those given *trans*-anethole on days 6-10 of pregnancy three out of five rats failed to deliver at term. No gross malformations of offspring were observed in any of the groups. The results demonstrated that *trans*-anethole has antifertility activity. From comparison with the days 1-2 group (lack of antizygotic activity), the lower level of delivery in the days 6-10 group was interpreted as a sign of early abortifacient activity (Dhar, 1995).

A well-established feto-placental co-culture system has been used to investigate *in vitro* the potential effects of *trans*-anethole and oestradiol on steroidogenesis. The fetal compartment (adrenal zone and liver) was represented by H295R human adrenocortical carcinoma cells and BeWo human placental choriocarcinoma cells were used to represent the placental trophoblast compartment. After a 24 h exposure of the co-culture to 2.5, 5.2 and 25 µM estragole or *trans*-anethole, hormone concentrations of oestradiol, estrone, dehydroepiandrosterone, androstenedione, progesterone and estriol were significantly increased. Using RT-qPCR, estragole and *trans*-anethole were shown to significantly alter the expression of several key steroidogenic enzymes, such as those involved in cholesterol transport and steroid hormone biosynthesis, including *StAR, CYP11A1, HSD3B1/2, SULT2A1, and HSD17B1, -4,* and *-5* (Yancu *et al.*, 2019a).

3.3.6. Local tolerance

Not applicable

3.3.7. Other special studies

No data available

3.3.8. Conclusions

Very limited and non-conclusive information on fennel toxicity derives from single and repeated-dose toxicity studies due to the limited number of studies and the small number of tested animals.

Aqueous and methanolic extracts of fennel did not show any mutagenic activity in the Ames test using *Salmonella typhimurium* strains TA 98 and TA 100, with or without S9-metabolic activation; in addition, methanolic extract of fennel (20–800 µg/mL) did not show genotoxic activity in Comet assay and micronucleus test after 24 h of treatment in HepG2 cells. Also, fennel powdered seeds at 10, 20 and 40 µg/mL did not show to be genoxotic in Comet assay after 4 h of treatment in HepG2 cells. The interpretation of the results of these studies is limited by methodological deficiencies (i.e., in the case of the Ames test, by the insufficient number of bacterial strains used, concentrations tested and replicates) or by the lack of sufficient information (i.e., in the case of the Comet assay). Moreover, information on the variety (*dulce* or *vulgare*) of fennel in these studies was missing.

In vitro and *in vivo* studies showed a weak mutagenic potential of anethole. However, taking into consideration the more recent results of the *Salmonella* tests repeated with the updated protocols as well as the results from the other genotoxicity studies it is considered that the positive response of anethole observed in the mouse lymphoma assay is most likely to be via a non-DNA mechanism. In addition, *trans*-anethole is reported as "generally recognised as safe" at the intake of 54 µg/kg bw per day) and the ADI is about 0-2 mg/kg bw based on JEFCA assessment (JEFCA, 1999).

There are no studies available on the carcinogenicity of fennel herbal substance or preparations. The genotoxicity and the carcinogenicity of estragole have been evaluated by HMPC and presented in the "Public Statement on the use of herbal medicinal products containing estragole" (EMA/HMPC/137212/2005 Rev 1 Corr 1*). Estragole metabolite 1'-hydroxyestragole was carcinogenic in rodents via DNA adduct formation. There is also evidence that under *in vivo* administration to humans, estragole can be metabolically activated to 1'-hydroxyestragole, probably in a similar way to rodents in which carcinogenicity has been observed. Therefore, the occurrence of the same mechanism for humans can be regarded as possible. (EMA/HMPC/137212/2005 Rev 1 Corr 1*).

Reproductive toxicity studies have shown that an acetone extract of fennel possess oestrogenic activity, which induces oestrus and increases oviductal uterine, cervical, vaginal and mammary gland weights in adult female rats. In addition, neonatal exposure to fennel ethanolic extract induces early vaginal opening and disrupts ovary function. On the other hand, the oestrogenic effect of hydro-alcoholic fennel seed extract resulted in reduced reproductivity and anti-fertility activity in male rats. No studies on fertility have been carried out using water extracts.

Trans-anethole, which is known to be a phytoestrogen, plays a relevant role in the anti-fertility effects of fennel as confirmed by studies carried out with fennel oil.

An aqueous extract of fennel seeds showed a dose-dependent teratogenic effect, which was more severe at oral concentrations of 12.5 and 25 mg of extract. The embryotoxic effect resulted in morphological changes, skeletal disorders, and cellular alterations.

Information on the variety (*dulce* or *vulgare*) of fennel extracts in reproductive toxicity studies was missing.

3.4. Overall conclusions on non-clinical data

The non-clinical data make plausible the traditional use of fennel fruit in mild spasmodic gastro-intestinal complaints, including bloating and flatulence, in spastic conditions and as an expectorant in cough associated with cold.

Aqueous extracts of fennel did not show any mutagenic activity in the Ames test using *Salmonella typhimurium* strains TA 98 and TA 100, with or without S9-metabolic activation. Also, fennel powdered seeds at 10, 20 and 40 µg/mL did not show to be genoxotic in Comet assay after 4 h of treatment in HepG2 cells. Methodology shortcomings and lack of adequate information limit the relevance of the results.

Results from studies carried out in the laboratory animals showed a weak mutagenic potential of anethole. *Trans*-anethole is reported as "generally recognised as safe" at the intake of 54 µg/kg bw per day) and the ADI is about 0-2 mg/kg bw (JEFCA, 1999). Zeller & Rychlik (2006) have experimentally determined an extraction rate of 16% for *trans*-anethole from bitter fennel fruits to teas. Taking into account the content of essential oil reported in literature for bitter and sweet fennel fruits (Raal *et al.*, 2012; Mihats *et al.*, 2016; Telci *et al.*, 2019), it is not expected that the daily intake of *trans*-anethole would be above the ADI set by JEFCA when comminuted sweet and bitter fennel fruits are taken as they are or as herbal teas according to the posologies reported in the monograph.

There is no evidence of genotoxicity for bitter and sweet fennel fruits and their preparations; however, the available data are too poor to draw any conclusion due to the methodology deficiencies of the studies or to the lack of sufficient information. In addition, adequate carcinogenicity data are missing. On the other hand, studies have shown the carcinogenic effects of estragole and 1'-hydroxyestragole in mice and rats (liver tumours). Although toxicokinetics and metabolism of estragole have not been thoroughly studied in humans, there is evidence that under *in vivo* administration of estragole to humans, the liver is exposed to the compound and the first step in metabolic activation, the formation of 1'-hydroxyestragole, is possible. Thus, it is probable that toxicokinetic processes in humans are similar to those in rodents in which carcinogenicity has been observed, and extrapolation can be regarded as possible. Therefore, the HMPC assessment in the "Public statement on the use of herbal medicinal products containing estragole" (EMA/HMPC/137212/2005 Rev 1 Corr 1*) concluded that the intake of estragole from HMPs in the general population should be as low as possible, which includes a short-time duration of use (maximum 14 days) and a discussion about the single/daily doses necessary according to the risk assessment relevant for the concerned HMP. For example, to reach or come as close as possible to the guidance value of 0.05 mg/person per day, the lowest dose should be consistently selected if ranges of single and daily doses are available from traditional use. Furthermore, 'low estragole plant varieties' should be recommended or a calculated adequate limitation of the estragole content in the specifications of the herbal medicinal products should be made.

This implies that in case of adults and adolescents, despite of evidence of long-standing use for doses of 1.5 g and 2.5 g of bitter and sweet fennel fruits with 0.25 L of boiling water three times daily as a herbal tea, only the lower dose will be included in the monograph. This corresponds to a daily dose of 4.5 g (1.5 g x 3 times daily).

Similarly, for children between 4-12 years of age, only the lower dose of 1.0 g of fruits with 0.25 L of boiling water three times daily as a herbal tea has been included in the monograph. This corresponds to a daily dose of 3.0 g.

For children, the HMPC assessment in the "Public statement on the use of herbal medicinal products containing estragole" (EMA/HMPC/137212/2005 Rev 1 Corr 1*) concluded that the usage of estragole

containing HMPs in children is not recommended if the daily intake of estragole exceeds the guidance value of 1.0 µg/kg bw, unless otherwise justified by a risk assessment based on adequate safety data. Hence, for children under 12 years of age the use is not recommended if the daily intake of estragole exceeds the guidance value of 1.0 µg/kg bw, unless otherwise justified by a risk assessment based on adequate safety data.

The body of the data indicates that reproductive system is a target for the action of fennel extracts, and their principal constituent *trans*-anethole, which may cause changes in male and female organs and tissues involved directly or indirectly in the reproductive mechanisms. Consequences of these changes are not easily predictable or detectable in humans. In addition, most of the reproductive toxicity studies were carried out on a limited number of animals tested and with ethanol or acetone extracts of fennel, which are herbal preparations not included in monograph. NOAELs were not determined in these studies.

An aqueous extract of fennel seeds showed a dose-dependent teratogenic, but further studies are needed to better investigate this effect.

In any case, the HMPC assessment in the "Public statement on the use of herbal medicinal products containing estragole" (EMA/HMPC/137212/2005 Rev 1 Corr 1*) concluded that the usage of estragole containing HMPs in pregnant and breast-feeding women is not recommended if the daily intake of estragole exceeds the guidance value of 0.05 mg/person/day.

4. Clinical Data

4.1. Clinical pharmacology

4.1.1. Overview of pharmacodynamic data regarding the herbal substance(s)/preparation(s) including data on relevant constituents

No data available.

4.1.2. Overview of pharmacokinetic data regarding the herbal substance(s)/preparation(s) including data on relevant constituents

No data available for fennel in humans. Indeed, only few *in vitro* studies on the inhibition of human CYP450 have been carried out.

An aqueous extract of fennel seed (50 mg/mL) inhibited CYP3A4 by over 90% and CYP3A7 by over 80%; the percentage of inhibition fallen to less than 60% towards CYP3A5 and less than 50% towards CYP2D6 isoenzymes (Nguyen *et al.*, 2014).

In another experiment, an aqueous extract of fennel seeds showed high inhibition of CYP2D6 and CYP3A4 with an IC_{50} constant of 23 ± 2 µg/mL and 40±4 µg/mL, respectively; less inhibition was seen towards CYP1A2 (IC_{50}: 115 ± 11 µg/mL). Fennel tea extracted with boiling water fully inhibited CYP2E1 activity *in vitro* within the investigated range of concentrations (1-1000 µg/mL). IC_{50} constants and IC_{50}/IC_{25} ratios were 23 ± 4 µg/mL and 2.29, respectively (Langhammer & Nilsen, 2013 and 2014).

To date very little is known about the metabolism of *trans*-anethole by humans. Caldwell's research group published two articles on metabolism of *trans*-anethole in humans, both including essentially the same experiments (Sangster *et al.*, 1987; Caldwell & Sutton, 1988). The fundamental conclusion of the authors regarding these experiments is only that "the pattern of urinary metabolites of trans-anethole

is unaffected by dose size". Any consideration on the risk influence is lacking. These Caldwell's experiments show essentially the difference in anethole metabolism between rodents and humans.

After oral administration of radioactively-labelled *trans*-anethole (as the methoxy-^{14}C compound) to five healthy volunteers at dose levels of 1, 50 and 250 mg on separate occasions, it was rapidly absorbed. 54-69% of the dose (detected as ^{14}C) was eliminated in the urine and 13-17% in exhaled carbon dioxide; it was not detected in the faeces. The bulk of elimination occurred within 8 h and, irrespective of the dose level, the principal metabolite (more than 90% of urinary-^{14}C) was 4-methoxyhippuric acid (Caldwell & Sutton, 1988). *Trans*-anethole is metabolised in part to the inactive metabolite 4-methoxybenzoic acid (Schulz *et al.*, 1998). An earlier study with two healthy subjects taking 1 mg of *trans*-anethole gave similar results (Sangster *et al.* 1987).

In humans, anethole is mainly metabolised to anisic acid, p-hydroxybenzoic acid, and 4-methoxy-hippuric acid. Anethole metabolites are eliminated within 8 h after anethole administration, and the major routes include renal excretion (54–69 %) and exhalation (13–17 %) (Aprotosoaie *et al.*, 2016).

A more recent study on healthy volunteers taking 500 mL of fennel tea containing 2.2 mg of estragole revealed the presence in urine samples of N-acetyl-S-[3′-(4-methoxyphenyl)allyl]-L-Cys (AMPAC), a mercapturic acid. This metabolite is supposed to be the product of detoxification of 1'-sulfooxyestragole by gluthatione conjugation. Before drinking the tea, the urinary AMPAC concentration was below the limit of detection. In most of the participants, the highest amounts of urinary AMPAC were found in the first-hour urine after exposure. The excretion by first-order kinetics (range of $t_{1/2}$ = 0.78−1.54 h; mean ± SD: 1.13 ± 0.21 h) led to a nearly complete clearance within 8 h in all participants. The total AMPAC excreted was in the range of 93−1076 ng, reflecting pronounced interindividual variations of enzymes taking part in estragole metabolism. Importantly, AMPAC was also formed in one volunteer following oral uptake of a single dose of isolated trans-anethole, albeit to a much smaller extent compared to estragole. (Monien *et al.*, 2019).

4.2. Clinical efficacy

4.2.1. Dose response studies

No data available.

4.2.2. Clinical studies (case studies and clinical trials)

- *Primary dysmenorrhoea*

Pattanittum *et al.* (2016) carried out a systematic review to determine the efficacy and safety of dietary supplements for treating dysmenorrhoea. The authors included parallel group or crossover randomised controlled trials (RCTs) of dietary supplements for moderate or severe primary or secondary dysmenorrhoea. They excluded studies of women with an intrauterine device. The effectiveness was evaluated using pain scores (all on a visual analogue scale (VAS) 0 to 10 point scale) or rates of pain relief, or both, at the first post-treatment follow-up. Four RCTs with fennel were included: fennel extract (46 mg) versus placebo every six hours (Moslemi, 2012), fennel capsule 30 mg every four hours versus no treatment (Ghodsi 2014), fennel oil 1% or 2% (0.3 to 1 mL) versus placebo, as required no more than four-hourly (Khorshidi 2003), and fennel 20 to 30 drops every four to eight hours versus placebo (Nazarpour, 2007). Also, clinical trials comparing fennel versus NSAIDs were identified: fennel 20 to 30 drops every four to eight hours versus mefenamic acid 250 mg every six hours (Nazarpour, 2007) and fennel 2% versus mefenamic acid 250 mg (Bokaie *et al*., 2013).

Finally, two clinical studies which compared fennel to vitamin E were included: fennel extract (46 mg) versus vitamin E (100 units) every six hours (Moslemi, 2012) and fennel extract (60 mg) versus vitamin E (150 IU), four times a day (Nasehi, 2013). For treating primary dysmenorrhoea, there was no consistent evidence of effectiveness for fennel. When fennel was compared to NSAIDs, there was no evidence of a difference. In addtion to the lack of high methodological quality of the studies included (e.g., small sample sizes, failure to report study methods) the authors pointed out that most included trials of primary dysmenorrhoea recruited university students. The applicability of the evidence to women in other contexts is uncertain (Pattanittum *et al.*, 2016).

Recently a further systematic review supported by a meta-analysis included four clinical trials which assessed the effect of fennel on the amount of menstrual bleeding: 46 mg of hydroalcoholic extract of fennel seed, or placebo, daily 5 capsules during the first three days of menstruation (Akhavan Amjadi, 2010), 25 drops of oral fennel drop 2% or no treatment (Bokaie *et al*., 2013), fennel oil 1% or 2% (0.3 to 1 mL) versus placebo, as required no more than four-hourly (Khorshidi, 2003), and 30 drops of fennel every 4 hours, 40 drops of *Vitex agnus-castus* every 4 hours, 250 mg of mefenamic acid or placebo (Shobeiri 2014). Meta-analysis results showed that using fennel significantly increases mean menstrual bleeding in the first cycle after treatment in the intervention group compared to the control (standard mean difference: 0.46; 95 % CI: 0.18–0.73; p=0.001). Of these four articles, only those conducted by Akhavan Amjadi *et al*. and Shobeiri *et al.* had assessed the amount of bleeding in the second cycle after treatment, and therefore, only these two articles were included meta-analysis for assessing the effect of fennel on menstrual bleeding in the second cycle after treatment. Fennel has no significant effect on the amount of menstrual bleeding in the second cycle after treatment in the intervention group compared to the control (mean difference: 1.44; 95 % CI: −5.09 to 7.96; p=0.67). The authors admitted the poor quality of articles and concluded that further clinical trials are necessary to determine the effect of fennel on menstrual bleeding (Abdollahi *et al.*, 2018).

- *Clinical studies in post-menopausal women*

The effect on vaginal atrophy was further investigated in a group of 60 post-menopausal Iranian women in a double-blind randomised controlled trial. The study participants were randomly divided into one of two groups, receiving either a placebo (n=30) or fennel 5% vaginal cream (n=30) administered as one application per day (5 g/day) for 8 weeks. In order to make an extract of fennel, fennel seeds were mixed with ethanol 80% and stored for 72 h. The extract was then dried using a rotary and freeze dryer. The vaginal pH and maturation vaginal index (MVI) were measured at baseline and 8 weeks after the intervention, while the vaginal atrophy symptoms were measured at baseline and at 2, 4, and 8 weeks after the intervention. The number of superficial cells increased in the fennel group after 8 weeks as compared to the control group (76.1 ± 15.3 vs.11.8 ± 8, p<0.001); the number of intermediate and parabasal cells decreased significantly in the fennel group as compared to the control group (p<0.001). The vaginal pH decreased significantly at the 8-week follow-up in the fennel group compared to the control group (100%vs. 7.4%, p<0.001). All women in the fennel group had an MVI of 65–100 at the 8-week follow-up, whereas almost half (40.7%) of the women in the control group had an MVI of 50–64 (p<0.001) (Yaralizadeh *et al.*, 2016).

Abedi *et al.* (2018b) assessed the effect of fennel on sexual function in 60 post-menopausal Iranian women with sexual dysfunction who were randomly assigned to two groups receiving either vaginal cream containing a dry ethanolic (ethanol 80%) extract of fennel seeds (n=30) or placebo (n=30). Vaginal atrophy in the women was assessed using symptoms such as pallor, dryness, dyspareunia, itching and burning. The pH of the vagina and cytology of the vaginal mucosa were also measured at baseline and 8 weeks after the intervention. All participants were requested to fill out the Female Sexual Function Index (FSFI) at baseline and after 8 weeks. The intervention group was requested to

use fennel vaginal cream (5 grams) every night, while the control group used placebo each night for 8 weeks. Sexual desire increased in the fennel and control groups significantly after 8 weeks (from 2 ± 0.53 to 5.3±.36 in the fennel group, $p<0.001$; from 1.8±0.6 to 2.9±0.46 in the control group, $p<0.001$). Desire increased in the fennel group more than it did in the control group ($p<0.001$). Other domains of sexual function (arousal, lubrication, orgasm, sexual satisfaction and pain) increased significantly in each group after 8 weeks. However, the fennel group showed significant improvement in all areas ($p<0.001$). The total FSFI score was significantly higher in the fennel group compared to the control group (8.2 ±9.4 and 8.03±10.36 before the intervention and changing to 33.79±0.7 and 18.99±1.09 after the intervention in the fennel and placebo groups, respectively; $p<0.001$) (Abedi *et al.*, 2018).

- *Pre-menstrual syndrome*

Premenstrual syndrome (PMS) includes a number of psychological and physical symptoms, which occur cyclically in the luteal phase of the menstrual cycle.

A single-blind randomised clinical trial was carried out to compare the effects of *Echinophora platyloba* and fennel extracts on the PMS against placebo in 90 Iranian students with moderate to severe PMS. Students were randomly divided into three equal groups. The first group received *Echinophora platyloba* extract, the second group received fennel extracts (no further information available) and the third group received placebo. The severity of PMS was measured by Daily Record of Severity of Problems (DRSP) questionnaire at the end of the first and second menstrual cycles before the intervention and the results were compared with them after the intervention. This form determines the severity of PMS using five items, including anxiety, depressive, emotional, somatic and fluid and electrolyte retention symptoms. Based on the DRSP form, scores ranging from 0 to 4 were allocated to evaluate the severity of symptoms, with higher scores indicating more severe symptoms. There were not any significant differences in the means of premenstrual syndrome scores before the intervention among the three groups (100.8±22.1 in echinophora-platyloba group, 101.3±27.1 in fennel group and 104.3±19.5 in placebo group, $p>0.05$). The differences were significant after the intervention (49.7±23.2 in *Echinophora platyloba* group, 64.4±27.5 in fennel group and 79.1±28.1 in placebo group, respectively, $p<0.001$). No significant differences were seen between the *Echinophora platyloba* and fennel groups (Delaram *et al.*, 2011).

The effects of regular exercise and fennel extract (no further information available) together and separately on PMS were compared in a randomised clinical trial on 48 Iranian students aged 16–18 years. Girls were selected by filling the DRSP questionnaire. The participants were divided into four equal groups: the first group received fennel, the second group had aerobic exercise, the third group received fennel along with exercise and the last group was control group without fennel and exercise. Participants filled DRSP-Q three times: the first menstrual cycle before the intervention, the first menstrual cycle after four weeks and finally the first menstrual cycle after eight weeks of intervention. After 8 weeks of intervention the severity of PMS symptoms reduced significantly in experimental groups (fennel, exercise and fennel + exercise) compared to control group ($p<0.05$). Meanwhile, there were not any significant differences in age, body mass index, age at menarche, age at dysmenorrhea onset and duration of menstruation among the four groups (Pazoki *et al.*, 2016).

- *Idiopathic hirsutism*

Idiopathic hirsutism is defined as the growth of androgen-sensitive hair in areas where these are naturally absent. It is called idiopathic when no reason is found for this complaint.

Thirty-eight women affected by idiopathic hirsutism were treated, in a double-blind placebo controlled study, for 12 weeks, twice a day, on their face with a cream containing 2%, 1% or 0% of fennel fruit extract obtained with ethanol in a Soxhlet apparatus for 5 hours. In both treatment groups (and more in the 2% fennel fruit extract), a significant reduction in facial hair growth and diameter was observed (Javidnia *et al*., 2003).

A randomised, double-blind, placebo-controlled clinical trial was carried out to evaluate the effect of fennel topical gel on mild to moderate idiopathic hirsutism in 44 Iranian women, randomly divided to case and control groups, each group included 22 cases. Exclusion criteria were: increased serum androgen level, irregular menstrual cycle, sever hirsutism, history of using spironolactone, cyproterone acetate, cyproterone compound, corticosteroids, medroxy progesterone acetate, contraceptive pills. Also, pregnant and lactating women, as well as patients who used Laser therapy for hair depilation during the previous 6 months were excluded. The case group received fennel gel 3% and the control group received placebo. The gel contained a dry extract obtained by maceration of fennel seeds using ethanol 80% as an extraction solvent. The effect of fennel gel 3% was defined as reduction of thickness of facial hair in micrometer by microscope in comparison with placebo. Measurements were performed at zero time and 24 weeks after treatment. The mean age of the patients in the case group was 26.9±6.7 and in the control group was 25.6±4.3 years. Hair thickness was similar between the two groups before intervention. The hair thickness reduced from 97.9±31.5 to 75.6±26.7 micron in patients receiving fennel gel after 24 weeks ($p<0.01$) (Akha *et al.*, 2014).

- *Milk production (galactogue)*

Foong *et al.* (2020) carried out a systematic review to assess the effect of oral galactagogues for increasing milk production in non-hospitalised breastfeeding mother-term infant pairs. Twenty-seven studies compared natural oral galactagogues (banana flower, fennel, fenugreek, ginger, ixbut, levant cotton, moringa, palm dates, pork knuckle, shatavari, silymarin, torbangun leaves or other natural mixtures) with placebo or no treatment. Three studies (fennel, fenugreek, moringa, mixed botanical tea) reported infant weight but could not be meta-analysed due to substantial clinical and statistical heterogeneity ($I2 = 60\%$, 275 participants, very low-certainty evidence). Based on subgroup analysis, the authors were very uncertain whether fennel or fenugreek improved infant weight, whereas moringa and mixed botanical tea might increase infant weight compared to placebo, although the magnitude of the effect was uncertain due to substantial heterogeneity of the studies, imprecision of measurements and incomplete reporting. In a study (Ghasemi 2018) fennel tea (7.5 g of fennel seed powder in black tea 3 times a day for 4 week) sand fenugreek tea (7.5 g of fenugrek seed powder 7.5 g in black tea 3 times a day for 4 weeks) were compared to determinate the effect on infant weight (Iranian girl infants with 0-4 months of age) where the infants received only own mother's milk. There was no difference in infant weight with fenugreek tea compared to fennel tea at one month (MD -10.25, 95% CI -462.91 to 442.41; 1 study, 78 participants; very low-certainty evidence). Another study (Matthew 2018) compared fennel tea (14 grams of in two litres of water, and 300 mL of this was given per day for seven consecutive days; n=15) and fenugreek tea (14 grams of in two litres of water, and 300 mL of this was given per day for seven consecutive days; n=15): the authors reported mean pre- and post- "lactational levels" for each group (Indian lactating women) but did not present a measure of dispersion (Foong *et al.*, 2020).

- *Post-partum pain*

A single-blind, clinical trial was conducted on 86 mothers with post-partum pain after vaginal delivery at Baharloo Hospital in Tehran, Iran to compare the effects of fennel extracts (no further information available) and mefenamic acid on postpartum pain. Subjects were randomly divided into two groups

(43 cases per group), receiving fennel extract and mefenamic acid capsules. The exclusion criteria were severe bleeding, long, difficult labour, prior history of gastrointestinal laceration or bleeding, cardiovascular diseases and unwillingness to continue the study. Postpartum pain was measured two hours after childbirth, using Visual Analogue Scale (VAS). Volunteers with scores higher than four were included in the study. Pain intensity was measured by VAS before and one hour after each round of intervention. Subjects used the medicines four times a day (with 4-6 hour intervals). The mean score of pain before the intervention was 6.47±0.797 in the mefenamic acid group and 6.35±0.752 in the fennel group. In both groups, pain score significantly reduced one hour after using medicines in comparison with the pre-treatment period (1.90±0.56 and 1.70±0.74 in the mefenamic acid and fennel groups, respectively) ($p<0.05$). However, no statistical difference was observed between the two groups. Before the final round of intervention, the mean pain score was 5.35±0.84 in the mefenamic acid group and 4.95±0.79 in the fennel group ($p=0.027$). In addition, the mean pain score one hour after the fourth round of intervention was 1.19±0.63 in the mefenamic acid group and 0.88±0.70 in the fennel group ($p=0.037$) (Golian Tehrani *et al.*, 2015).

Table 7: Clinical studies on humans, in post-menopausal women

Type	**Study**	**Test product(s):**	**Number of subjects**	**Healthy subjects**	**Outcomes (primary and secondary endpoints)**	**Statistical analysis**	**Comments**
To investigate the effect of fennel on vaginal atrophy in post-menopausal (PM) women. Yaralizadeh *et al*. 2016	Double-blind randomised placebo-controlled trial Primary outcome measures: 1) vaginal pH and maturation vaginal index (MVI) at baseline and 8 weeks after the intervention; 2) vaginal atrophy symptoms measured at baseline and at 2, 4, and 8 weeks after the intervention	Placebo or vaginal cream containing 5% of dry ethanolic extract of fennel seeds, one application per day (5 g/day) for 8 weeks	60 PM women randomly assigned into two groups receiving either fennel 5 (n=30) or placebo (n=30). The mean age of the participants in the intervention and control groups was 53.73 ± 3.6 and 52.90 ± 3.4 years in fennel group and placebo group, respectively. No participants withdrew dur-ing the study or prior to the 8-week follow-up.	Inclusion criteria: 1) participants aged between 45 and 65 years with natural menopause confirmed by amenorrhea for at least 12 months or by the elevation of FSH and LH in laboratory tests; 2) had symptoms of vaginal atrophy; 3) engaged in sexual activity; 4) monogamous. Exclusion criteria: vaginal infection, hormonal use during the 8 weeks prior to the study, smoking, alcohol use, uterine bleeding from unknown causes, and phytoestrogen	Number of superficial cells increased in the fennel group after 8 weeks as compared to the control group (76.1 ± 15.3 vs. 11.8 ± 8, p<0.001). Number of intermediate and parabasal cells decreased significantly in the fennel group as compared to the control group (p<0.001). Significant improvements with fennel in symptoms, including itching (p=0.017), dryness (p<0.001), pallor (p<0.001), and dyspareunia (p<0.001). Burning symptoms improved with fennel, but difference was not significant compared to	All data were entered using the SPSS version 22. The data were analysed using the independent t-test, chi-squaretest, paired sample t-test, and the generalised estimating equation	Short duration of the study and small sample size limited the relevance of the observed results. Serum levels of estrogen were not determined

Type	Study	Test product(s):	Number of subjects	Healthy subjects	Outcomes (primary and secondary endpoints)	Statistical analysis	Comments
				use in the past month	placebo (p=0.14). Significant decrease in vaginal pH with fennel at the 8-week follow-up compared to the control (100% vs. 7.4%, p<0.001)		

Type	Study	Test product(s):	Number of subjects	Healthy subjects	Outcomes (primary and secondary endpoints)	Statistical analysis	Comments
To assess the effect of fennel on sexual function in post-menopausal women. Abedi *et al*. 2018	Double-blind randomised placebo-controlled trial Primary outcome measure: score of Female Sexual Function Index (FSFI) at baseline and after 8 weeks	Vaginal cream containing a dry ethanolic (ethanol 80%) extract of fennel seeds (n=30) or placebo (n=30)	60 post-menopausal Iranian women with sexual dysfunction The mean age of women in the fennel group was 53.73±3.6 and 52.9±3.4 years in the control group (p>0.05). Drop-outs: none	Inclusion criteria: women with age between 45 and 65 years, women who had natural menopause confirmed by amenorrhea for one year or increased follicle stimulation hormone (FSH)≥40 IU/l, women who lived with their husband and were sexually active, and score of Female Sexual Function Scale <26	The total FSFI score was significantly higher in the fennel group compared to the control group after the intervention (8.2 ± 9.4 and 8.03±10.36 before intervention and 33.79±0.7 and 18.99±1.09 after the intervention in the fennel and placebo groups, respectively; p<0.001). Desire increased in the fennel and control groups significantly after 8 weeks (from 2 ± 0.53 to 5.3±.36 in the fennel group, p<0.001; from 1.8±0.6 to 2.9±0.46 in the control group, p<0.001). Other domains of sexual function increased significantly in each	All data were entered into SPSS, version 22. The independent t-test was used to compare the two groups regarding continuous data. Mann-Whitney U test and Wilcoxon signed-ranked test was used to compare sexual function before and after the intervention	Short duration of the study and small sample size limited the relevance of the observed results

Type	Study	Test product(s):	Number of subjects	Healthy subjects	Outcomes (primary and secondary endpoints)	Statistical analysis	Comments
					group after 8 weeks; the fennel group showed significant improvement in all areas ($p<0.001$)		

Table 8: Clinical studies on humans, in pre-menstrual syndrome

Type (aim) and Objective(s) of study; Reference	**Study design and Type of Control Study duration (if available)**	**Test Product(s): Herbal preparation, Pharmaceutical form; Dosage regimen; Route of administration Duration of treatment**	**Number of subjects (including age, sex, drop out)**	**Healthy subjects or Diagnosis of patients (inclusion criteria)**	**Outcomes (primary and secondary endpoints)**	**Statistical analysis (e.g. ITT yes/no, CI 95%); Quality score (e.g. Jadad score)**	**Comments on clinical relevance of results**
To compare the effects of echinophora-platyloba and fennel against placebo on premenstrual syndrome Delaram *et al*., 2011	Single-blind placebo controlled clinical trial Primary outcome measure: decrease in total premenstrual syndrome scores determined by filling Daily Record of Severity of Problem (DRSP) at the end of the first and second menstrual cycles	30 drops of echinophora-platyloba extract, fennel extract or placebo (sterile water) every 8 hours, 3 days before until 3 days after the onset of menstrual bleeding	90 women aged 18-25 years randomly assigned to each group (n=30) Nine drop-out (4 in echinophoraplatyloba group, 2 in fennel group and 3 in the placebo group)	90 women selected having highest PMS based on filling DRSP among 250 students. Exclusion criteria: pelvic inflammatory disease, any chronic diseases, drug use or with any stressor factors	The total premenstrual syndrome scores have been reduced upon the treatments in three groups after the intervention ($p<0.001$). The effects of the two extracts were more significant than these of placebo ($p=0.009$)	Data was analysed by Dunn, Kruskal-Wallis and Pearson correlation tests, using SPSS	Limitation: small sample size, study not double-blinded, short duration of therapy

Type (aim) and Objective(s) of study; Reference	**Study design and Type of Control Study duration (if available)**	**Test Product(s): Herbal preparation, Pharmaceutical form; Dosage regimen; Route of administration Duration of treatment**	**Number of subjects (including age, sex, drop out)**	**Healthy subjects or Diagnosis of patients (inclusion criteria)**	**Outcomes (primary and secondary endpoints)**	**Statistical analysis (e.g. ITT yes/no, CI 95%); Quality score (e.g. Jadad score)**	**Comments on clinical relevance of results**
	before the intervention and the results were compared with them after the intervention						
To compare the effects of regular exercise and fennel together and separately on PMS in high school girls Pazoki *et al*., 2016	Randomised clinical trial Primary outcome measure: decrease in total premenstrual syndrome scores determined by filling Daily Record of Severity of Problem (DRSP).	30 drops, every 8 h, 3 days before until 3 days after the onset of menstrual bleeding. The aerobic exercise program consisted of a ten-min. warm-up, 40 min of fast exercise in limb and trunk followed	48 female high school students aged 16–18 randomly assigned to four equal groups: 1) aerobic exercise, 2) fennel extract, 3) fennel extract + exercise and 4) control (did not	48 women selected having moderate (the individual has problems in maintaining daily activities, but can go to work or school) or severe (the individual is not able to do daily activities) PMS based on filling DRSP among 200 students	Fennel, regular aerobic exercise and their combination significantly reduced the severity of PMS symptoms compared to control group both at middle of intervention and after 8 weeks of intervention ($p<0.05$)	Data were analysed by ANOVA and paired T-test in SPSS (v.16)	Limitations: small sample size, study not blinded, inclusion/exclusion criteria not reported, short duration of therapy.

Type (aim) and Objective(s) of study; Reference	Study design and Type of Control Study duration (if available)	Test Product(s): Herbal preparation, Pharmaceutical form; Dosage regimen; Route of administration Duration of treatment	Number of subjects (including age, sex, drop out)	Healthy subjects or Diagnosis of patients (inclusion criteria)	Outcomes (primary and secondary endpoints)	Statistical analysis (e.g. ITT yes/no, CI 95%); Quality score (e.g. Jadad score)	Comments on clinical relevance of results
	PMS score was determined the first menstrual cycle before the intervention, 2 – the first menstrual cycle after four weeks of aerobic exercise and using fennel and finally first menstrual cycle after 8 weeks of intervention	by 10 min cooldown	receive fennel or exercise)				

Table 9: Clinical studies on humans, in idiopathic hirsutism

Type (aim) and Objective(s) of study; Reference	**Study design and Type of Control Study duration (if available)**	**Test product(s): Herbal preparation; Pharmaceutical form; Dosage regimen; Route of administration; Duration of treatment**	**Number of subjects (including age, sex, drop out)**	**Healthy subjects or Diagnosis of patients (inclusion criteria)**	**Outcomes (primary and secondary endpoints)**	**Statistical analysis (e.g. ITT yes/no, CI 95%); Quality score (e.g. Jadad score)**	**Comments on clinical relevance of results**
Treatment of hair thickness Javidnia *et al*., 2003	Double-blind placebo controlled clinical trial Primary endpoint: reduction in hair growth (decreased frequency of epilation) assessed by modified Ferriman-Gallwey method	Cream containing 2%, 1% or 0% of fennel fruit ethanolic extract or placebo applied on the face twice a day for 12 weeks	45 women Seven drop-out (4 in group receiving cream containing 1% fennel extract and 3 in placebo group)	Female patients aged 16–53 years (mean age 29 years) with mild to moderate forms of idiopathic hirsutism localised to the face. None had polycystic ovaries and all of them had serum androgen levels within the normal range	In both treatment groups (and more in the 2% fennel fruit extract), a significant reduction in facial hair growth and diameter was observed odour ($p=0.000524$ 2% vs 1% of fennel extract; $p=8.05\times10^{-5}$ 1% fennel extract vs placebo; $p=4.96\times10^{-7}$ 2% fennel extract vs placebo)	No information reported	Limitations: small sample size, no info on statistical analysis, high rate of drop-out

Type (aim) and Objective(s) of study; Reference	**Study design and Type of Control Study duration (if available)**	**Test product(s): Herbal preparation; Pharmaceutical form; Dosage regimen; Route of administration; Duration of treatment**	**Number of subjects (including age, sex, drop out)**	**Healthy subjects or Diagnosis of patients (inclusion criteria)**	**Outcomes (primary and secondary endpoints)**	**Statistical analysis (e.g. ITT yes/no, CI 95%); Quality score (e.g. Jadad score)**	**Comments on clinical relevance of results**
To evaluate the effect of fennel topical gel on mild to moderate idiopathic hirsutism. Akha *et al.*, 2014	Double-blind placebo controlled randomised clinical trial Primary outcome: the effect in the study group defined as reduction of thickness of facial hair in micrometer by microscope at 0 time and after 24 weeks of treatment in	Study group : gel containing 3% dried fennel extract (obtained by macerating twice the powdered fennel seeds in ethanol 80%, no further specification) for 24 weeks Control group: same gel formulation without the fennel extract.	44 women randomly divided in 2 groups of 22 (study and control). Mean age of patients was 26.9±6.7 and 25.6±4.3 years in study and control groups, respectively. Drop out: 2 patients in the control group	Patients with mild to moderate idiopathic hirsutism limited to face (upper lip, check and chin) and aged 15- 45 years old. Exclusion criteria were increased serum androgen level, irregular menstrual cycle, severe hirsutism, history of using spironolactone, cyproterone acetate, cyproterone compound, corticosteroids, medroxy progesterone acetate, contraceptive pills;	Primary outcome: Reduction of hair thickness from 97.9±31.5 to 75.6±26.7 µm in study group after 24 weeks ($p<0.01$)	Study power of 80%. Paired t-test for comparison of hair thickness changes in groups; Student t-test for comparison of hair thickness difference between groups	Limitation: cases with severe hirsutism were not included; small sample size

Type (aim) and Objective(s) of study; Reference	Study design and Type of Control Study duration (if available)	Test product(s): Herbal preparation; Pharmaceutical form; Dosage regimen; Route of administration; Duration of treatment	Number of subjects (including age, sex, drop out)	Healthy subjects or Diagnosis of patients (inclusion criteria)	Outcomes (primary and secondary endpoints)	Statistical analysis (e.g. ITT yes/no, CI 95%); Quality score (e.g. Jadad score)	Comments on clinical relevance of results
	comparison with placebo			pregnant and lactating women, patients who used laser therapy for hair depilation during the previous 6 months			

Table 10: Clinical studies on humans, in post-partum pain

Type (aim) and Objective(s) of study; Reference	**Study design and Type of Control Study duration (if available)**	**Test product(s): Herbal preparation; Pharmaceutical form; Dosage regimen; Route of administration; Duration of treatment**	**Number of subjects (including age, sex, drop out)**	**Healthy subjects or Diagnosis of patients (inclusion criteria)**	**Outcomes (primary and secondary endpoints)**	**Statistical analysis (e.g. ITT yes/no, CI 95%); Quality score (e.g. Jadad score)**	**Comments on clinical relevance of results**
To compare the effects of fennel extracts and mefenamic acid on postpartum pain Golian-Tehrani *et al*., 2015	Single-blind, clinical trial Primary outcome measure: reduction in VAS score after each intervention	Subjects used fennel capsules or mefenamic acid capsules Postpartum pain was measured two hours after childbirth, using VAS. Pain was measured one hour after taking study medications, The intervention continued for 24 hours after childbirth. During this period, both	86 mothers with medium or severe postpartum pain (VAS score 4 or higher) after vaginal delivery. Subjects were randomly divided into two groups (43 cases per group) Four drop-out (2 in each group)	Main inclusion criteria: 1) giving natural birth; 2) gestational age of 38-40 weeks; 3) singleton live birth; 4) fetal weight of 2500-4000 g; 5) no severe bleeding; 6) no sensitivity to prescribed medicines; 7) no drug addiction or dependence on psychotropic drugs; 8) absence of recognised diseases; 9) spontaneous	Pain score was 6.47±0.8 in the mefenamic acid group and 6.35±0.75 in the fennel group before the intervention. In both groups, pain score significantly reduced one hour after using medicines in comparison with the pre-treatment period (1.90±0.56 and 1.70±0.74 in the mefenamic acid and fennel groups, respectively) ($p<0.05$). During the study, fennel	Independent t-test, Chi-square and Fisher's exact test were performed, using SPSS version 18	Limitation: study not double blinded, no control group included, small sample size

Type (aim) and Objective(s) of study; Reference	Study design and Type of Control Study duration (if available)	Test product(s): Herbal preparation; Pharmaceutical form; Dosage regimen; Route of administration; Duration of treatment	Number of subjects (including age, sex, drop out)	Healthy subjects or Diagnosis of patients (inclusion criteria)	Outcomes (primary and secondary endpoints)	Statistical analysis (e.g. ITT yes/no, CI 95%); Quality score (e.g. Jadad score)	Comments on clinical relevance of results
		groups received the capsules four times within 4-6 hour intervals. The same procedure was performed for each round of intervention		rupture of membranes; 10) undergoing no interventions such as abortion or assisted vaginal delivery; 11) absence of grade III or IV tears. Main exclusion criteria: 1) severe bleeding; 2) history of GI laceration or bleeding; 3) cardiovascular diseases	was more effective than mefenamic acid in pain reduction. The same finding was reported after the study during the fourth round of intervention (88±0.70 and 1.19±0.76 in the fennel and mefenamic acid groups, respectively		

4.3. Clinical studies in special populations (e.g. elderly and children)

- *Anti-colic effect*

The efficacy of fennel tea for treating infantile colic was addressed by Weizman *et al*. (1993). A randomised, placebo-controlled study, carried out on 121 (62 in the treated group and 59 in the control group) infants between 2-12 weeks of age, suggested that an oral administration for 7 days of a 0.1% fennel seed oil emulsion in water (corresponding to about 12 mg/kg bw and day) is significantly superior ($p<0.01$) to placebo in decreasing intensity of infantile colic.

A randomised, double-blind, placebo-controlled trial was carried out to investigate the effectiveness and side effects of a phytotherapeutic agent based on powdered extracts of *Matricariae recutita* L., *Foeniculum vulgare* M. var. *dulce* and *Melissa officinalis* L. in the treatment of 93 breastfed colicky infants. The results showed that colicky infants treated with the extract improved within one week of treatment (Savino *et al*., 2005).

A Cochrane systematic revision to assess the effectiveness and safety of pain-relieving agents for reducing colic in infants younger than four months of age included also clinical studies carried out with fennel (Weizmann, 1993; Aleksandrovich, 2003; Arikan, 2008; Savino, 2005). Herbal agents (i.e., extract of *Matricaria recutita*, *Foeniculum vulgare* and *Melissa officinalis*; fennel seed emulsion; Fumaria extract; and herbal tea preparation) were associated with reductions in crying duration compared with placebo or no treatment, and with improvement in symptoms, compared with placebo. However, the quality of the evidence is low or moderate (Biagioli *et al.*, 2016).

- *Effect on growth*

Ghasemi *et al.* (2014) assessed the effect of herbal tea containing fennel seed on breast milk sufficiency signs and growth parameters of Iranian infants in medical health centres of Tehran University of Medical Sciences, Tehran, Iran. Seventy-eight girl infants aged 0-4 months who were exclusively breastfed, were randomly assigned into the intervention group (received herbal tea containing 7.5 g fennel seed powder in addition to 3 g black tea three times a day) and the control group (received herbal tea containing 3 g black tea powder three times a day). Before and during four weeks of study, signs of breast milk sufficiency were evaluated through measuring the growth parameters and the number of wet diapers in a day, frequency of defecation and infant breastfeeding times. Before intervention, there was no significant difference between weight, height, head circumference, the number of wet diapers and frequency of defecation times between the two groups ($p>0.05$), but the number of breastfeeding times of control group was more than the fennel group. After fourth weeks, compared to pre-intervention conditions, fennel significantly increased weight, head circumference, the number of wet diapers from, the frequency of defecation times and the number of breastfeeding times ($p<0.001$), but it had no effect on height ($p=0.066$) (Ghasemi *et al.* 2014).

4.4. Overall conclusions on clinical pharmacology and efficacy

There are no clinical studies relevant to the indications summarised in Tables 4 and 5; available clinical data concern medical uses other than of herbal medicinal products authorised in the EU countries.

In the clinical study investigated the effect of fennel on primary dysmenorrhea symptoms and menorrheal duration (Ghodsi & Asltoghiri, 2014) the herbal preparation was not reported. Significant reduction in nausea and weakness score as well as a descrease in menstruation duration was

observed, but fennel did not have any effect on decrease of bleeding amount. Due to several methological deficiences the clinical relevance of these studies is strongly limited and cannot be sufficient to support a well-established use.

Several clinical studies have been carried out to study the effect of fennel on post-menopausal symtoms. Most of these studies failed to show a significant effect of fennel compared to placebo. In addition, even when positive results were observed, the small sample size and the short duration of treatment were the shortcomings that limited the clinical relevance. Finally, in most cases the herbal substance/preparation used was not reported.

Two clinical studies investigated the effect of fennel extract (not specified) as oral drops in alleviating PMS symptoms. Although some positive results could be observed, the poor methodological design of these studies (i.e., small sample size, studied not blinded, short duration of treatment) did not allow any conclusion on the efficacy.

Two small clinical studies showed a significant effect of ethanolic extract of fennel seeds against idiopathic hirsutism versus placebo, but one of the studies did not include patients with severe hirsutism whilst the other was affected by a high rate of drop-out.

Investigations available in human beings on the role of fennel in reducing pain in infantile colic are very preliminary and only traditional use can be proposed (for safety aspects see section 5.5 Safety in special populations and situations).

In conclusion, the medicinal use of fennel fruit is not supported by adequate clinical evidence. On the basis of the long standing use reported in scientific literature (see section 2.1) traditional medicinal use can only be proposed.

5. Clinical Safety/Pharmacovigilance

5.1. Overview of toxicological/safety data from clinical trials in humans

No significant side effect has been reported in the few studies performed with fennel. A small number of patients topically treated with cream or gel containing ethanolic extract had irritation and/or itching. In a few clinical studies, Iranian post-menopausal women treated with oral capsules containing 30% fennel standardised to 21–27 mg anethole experienced mainly frequent urination and spotting; notably, there were two cases of vaginal bleeding.

A retrospective observational study to investigate the proportion, prevalence of use, attitude and knowledge base in a sample of Italian pregnant women in the south of Italy was conducted during the period November 2010–September 2013. Six hundred and thirty expectant mothers (31-40 years of age) were interviewed within three days after childbirth to explore the possible influence and risks of herbal consumption on pregnancy and neonatal outcomes. Fennel was among the most commonly used herbal products, taken by oral route and for the entire period of pregnancy by 94 women (15.7%). The following pregnancy and neonatal outcomes were considered in the study: the course of pregnancy (physiological or pathological course), abnormalities in foetal growth, type of labour (spontaneous or induced labour), gestational age, birth weight, small for gestational age, Apgar score, circumference of the skull and newborn's length. No side effects were reported after fennel consumption. A regular consumption of fennel throughout the pregnancy resulted in shorter gestational age compared to non-users (38.8 ± 2.2 weeks versus 39.1 ± 1.6 weeks; $p<0.05$). Moreover, the frequency of lower length of the newborn resulted relatively higher in users, although this did not reach statistical significance (49.5 ± 2.6 cm versus 49.9 ± 1.2 cm; $p=0.06$). None of the remaining

evaluated outcome variables were significantly influenced by the mother's consumption of fennel (Trabace *et al.*, 2015).

5.2. Patient exposure

No data available.

5.3. Adverse events, serious adverse events and deaths

A male premature baby 8 weeks old experienced hyponatraemic seizure following administration of fennel tea for abdominal pain (medical history: abdominal pain, upper respiratory attract infection, bronchiolitis, gastroesophageal reflux disease, irritability) (HPRA report identification number 2013-017369; onset date: 20/11/2012; recovered/resolved).

A case-report of hepatoxicity in two women, aged 26 and 30 years, who consumed a herbal tea containing fennel and cumin to increase lactation everyday for three-four weeks was published. They experienced symptoms of nausea, vomiting, anorexia and weakness. Serum alanine aminotransferase and aspartate aminotransferase levels were increased; all serological tests for viral hepatitis and autoimmune disorders were negative. The herbal tea was discontinued upon admission and the patient was treated conservatively. Frequent assessment of the liver function was performed. Liver enzymes returned to normal values four-five weeks later (Zengin *et al.*, 2014).

Türkyilmaz *et al.* (2008) reported four cases of premature thelarche in Turkish girls (aged between 5 months to 5 years) between January 2001 and Decembre 2007 after consumption of fennel tea 2 or 3 times a day for several months to eliminate gas pain. All of the infants had been breast-fed in the first nine months, and they had no history of prolonged drug intake that could cause premature thelarche. The physical examination and genital examination of the patients were normal. The serum oestradiol levels of all four patients were 15-20 times higher than the normal values for their age. Thus, all of the mothers were instructed to stop administering *Foeniculum vulgare* tea to their children. Premature telarche resolved within 3-6 months, and oestradiol levels returned to normal range.

A further case of a 12-month-old girl who showed breast development that became apparent in the last 3 months was reported more recently. Her medical history revealed that she was given two to three teaspoons of fennel tea by her mother every day for restlessness for the last 6 months. Isolated premature thelarche (breast development in the absence of other secondary sex characteristics in prepubertal girls) was diagnosed based on physical and laboratory findings. On follow-up, after cessation of fennel consumption, the breast development of the patient regressed gradually and became normal by the end of one year. Appearance of telarche was explained with use of fennel because its content of the phytoestrogen anethole as an active ingredient (Okdemir *et al*., 2014).

Allergic reactions to fennel, affecting the skin or the respiratory system, occur rarely (Levy, 1948; Schwartz *et al*., 1997; Blumenthal and Goldberg, 2000).

Enzyme immunoassay inhibition studies with one patient’s serum revealed cross-reactivity among the IgE components deriving from aniseed, fennel, caraway, coriander and dill extracts (Garcia Gonzalez *et al*., 2002).

A “mugwort-celeryspice-syndrome”, a pollen-food allergy that occurs in a minority of mugwort pollen-allergic was first reported more than 25 years ago. Reported offending foods include celery root, anise, fennel, coriander, cumin, pepper, and paprika patients. Borghesan *et al.* (2013) reported two cases of cross-reactivity between mugwort pollen and fennel. The first case was a 20 year old man with a

history of mild grass pollen allergy who experienced anaphylaxis a few min. after the ingestion of a small portion of raw fennel (generalised urticaria, dysphonia, lips angioedema, palm-plantar itch). The allergic reaction subsided at home after the administration of systemic steroids and oral cetirizine. On allergological assessment the patient showed strong skin reactivity to fresh raw fennel (mean wheal diameter 20 mm) and a moderate reactivity to fresh cooked fennel. Skin prick tests with a series of commercial food extracts (ALK-Abellò) scored positive for peanut, hazelnut, and peach, as did a SPT with fresh apple. SPT scored frankly positive for grass and mugwort pollen. The second case was a 41 year old man with a history of mild perennial rhinitis with seasonal worsening who experienced two episodes of oral allergic syndrome and dyspnoea few min. after ingestion of raw and cooked fennel, respectively. In both occurrences the allergic reaction subsides in two hours without therapy. On the allergological assessment, which was performed three months after the last adverse reaction, the patient showed strong skin reactivity to grass, mugwort, cypress, mites, Alternaria, cat's dander, and raw fennel. The authors suspected a 60 kDa allergen, highly homologous to Api g 5, recognised in fennel by patient's IgE, to be responsible for the mugwort-celery-spice syndrome (Borghesan *et al.*, 2013).

Nico *et al.* (2014, only abstract available) analysed a recent series of 189 well diagnosed cases of food allergy, with the purpose of estimating the occurrence of fennel allergy, in a population with a typically Mediterranean Diet, from Apulia – Southern Italy. For fennel, the investigation was carried out by quantitative skin prick tests with a commercial extract, quantitative prick by prick procedure with the fresh vegetable and CAP RAST for fennel. Allergy to fennel was clearly diagnosed in 57 patients (30% of all food allergy patients), 11 (19%) of whom were positive only for fennel. Many of these patients exhibited also multiple sensitisations to food allergens of the Apiaceae family. Thus 45 patients (79%) had positive skin tests for celery, 22 (39%) for parsley (Petroselinum crispum), and 21 (37%) for carrot (Daucus carota). Notably, all of these patients had lip angioedema and oral itching after fennel's ingestion. However, 17 (30%) had also Quincke's oedema, 11 (19%) urticaria and finally, one patient experienced severe anaphylaxis, after eating row fennel.

A recent case of an 11-year-old boy, presented to the specialist pediatric allergy clinic with a history of recurrent, immediate hypersensitivity reactions to a variety of toothpastes, in addition to curry, mint, liquorice, cauliflower, and broccoli over the last few years, has been recently published. The most troublesome reaction was to Kingfisher Fennel Natural Toothpaste, which the patient now avoids. The patient had no eczema or asthma, but he did have seasonal allergic rhinitis. He was not taking any medication. The findings from the examination of his skin, cardiovascular system, and lungs were normal. He had signs of nasal congestion but no nasal polyps. Serum specific IgE test result was positive to fennel (fresh fennel 10.5 kUA/L). Skin prick to prick testing to Kingfisher fennel-flavoured toothpaste had a positive result with a wheal of 12 mm. To confirm the clinical allergy to the toothpaste, a physician-supervised open challenge was performed. After being challenged with 200 mg of toothpaste, the patient immediately developed an itchy mouth and rhinorrhea, spat out the toothpaste and rinsed his mouth. Within a min., he started coughing but did not wheeze. His oxygen saturation and other vital signs remained within the reference range. He had no flushing, urticaria, angioedema, vomiting, or other gastrointestinal symptoms. All symptoms resolved within 10 min. without any treatment. On a separate occasion, an oral challenge to 100 ground fennel seeds was performed, to which patient showed no reaction. However, immediately after chewing fresh fennel root, the patient started to cough and his voice became hoarse, indicating that the allergen was present in the fennel root but not in the seeds (Denaxa & Arkwright, 2020). Interestingly, two cases of cheilitis and perioral dermatitis secondary to allergic contact dermatitis to limonene contained in toothpaste (Trokoudes & McFadden, 2016, only abstract available); limonene is a natural constituent in fennel essential oil.

Rare cases of contact dermatitis to anethole containing preparations (Andersen, 1978; Franks, 1998) have been reported.

It has been observed that fennel contains coumarin-derivatives, which competitively can inhibit vitamin K and may interfere with blood clotting (Shlosberg and Egyed, 1985). No further data are available.

Fennel contains small amounts of bergapten, a linear furocoumarin that might be responsible for phototoxicity (Kwon *et al*., 2002).

EudraVigilane database

In total, 91 reports were found in EudraVigilance database (search date: 04th of May 2021) using "Fennel" and "Foeniculum" as search terms, also including combinations. Out of these, 31 cases were serious whose ten resulted in hospitalisation and one was life-threatening. Most of the reports referred to polyherbal preparations, with fennel combined with at least other two plants; in particular, a product containing levomenthol, cinnamon oil, clove oil, eucalyptus oil, thymol, fennel oil, sage oil, star anise oil) accounts for 23% of the total reports. Adverse events more frequently reported concerns allergic reactions and epidermal/dermal conditions (hypersensitivity, angioedema, pruritus, rash, swelling of lips, tongue and face); two cases of Steven-Johnson syndrome (one life-threatening) were also reported. In a vast majority of reports, people recovered from these adverse events also when they were serious. Gastrointestinal disturbances were also frequently observed (abdominal pain, nausea, diarrhoea) and in all cases they were not serious. Three serious cases of renal adverse events (acute or chronic kidney injury and blood creatinine increased) which involved polyherbal preparations (including at least five plants apart fennel) were found; one case of acute hepatitis and one case of hepatic cytolysis with teas were reported with tablets containing Cassia angustifolia, Althaea Officinalis, *Foeniculum Vulgare* and with a herbal tea made of chamomile and fennel extracts, respectively were also reported. Causality assessment was not possible due to poor narrative, use of concomitant medication and absence of challenge/rechallenge.

Products containing fennel as monoingredient were involved in only nine reports. Four cases of premature telarche were reported in children assuming fennel tea for flatulence (Türkyilmaz *et al.*, 2008). One case of post-menopausal haemorrhage was observed in a female 65 year old. One case of alleric dermatitis which caused hospitalisation was also reported. All remaining reports were not serious.

5.4. Laboratory findings

No data available.

5.5. Safety in special populations and situations

5.5.1. Use in children and adolescents

For bitter and sweet fennel seeds preparations, there is evidence of traditional use for symptomatic treatment of mild, spasmodic gastro-intestinal complaints including bloating, and flatulence, as well as expectorant in cough associated with cold in children below 11 years of age when taken as herbal tea with a daily dose of 3-5 g. Cases of premature telarche have been reported in babies below four years who were administered fennel tea, although after several months of daily intake.

Based on the evidence of traditional use, sweet and bitter fennel should be taken as herbal tea by children and adolescents with a daily dose of 4.5-7 g and of 3-5 g, respectively. For children between

4-12 years of age the use is not recommended if the daily intake of estragole exceeds the guidance value of 1.0 µg/kg bw, unless otherwise justified by a risk assessment based on adequate safety data.

The use is not recommended in children under four years of age due to the lack of adequate data.

5.5.2. Contraindications

Hypersensitivity to the active substance or to Apiaceae (Umbelliferae) (aniseed, caraway, celery, coriander and dill) or to anethole. A cross-allergenicity between fennel and celery has been reported (Stager *et al*., 1991). A common allergen called Bet v 1 possibly accounting for the observed cross-sensitivity was found in subjects showing allergic symptoms as rhinitis, angioedema, asthma, wheezing, urticaria, eczema, abdominal pain, vomiting, and diarrhoea (Jensen-Jarolim *et al.*, 1997; Garcia-Gonzalez *et al.*, 2002).

Cross-reactivity with mugwort pollen has been also reported (Borghesan *et al.*, 2013), therefore the use of fennel should be contraindicated in case of known hypersensitivity to mugwort pollen.

5.5.3. Special Warnings and precautions for use

Patients should seek medical advice if symptoms persist for more than two weeks or worsen upon administration of the medicinal product.

5.5.4. Drug interactions and other forms of interaction

No case has been reported.

Fennel contains a high amount of minerals, mainly calcium, magnesium, iron, zinc, manganese, and copper. It has been shown in the rat that co-administration of fennel and ciprofloxacin may lead to decreased bioavailability of ciprofloxacin in rats due to formation of a ciprofloxacin-cation complex with possible decrease of ciprofloxacin efficacy. Formation of a ciprofloxacin-cation complex resulted in reduced ciprofloxacin absorption. Co-administration of ciprofloxacin with fennel led to a 83% reduction in ciprofloxacin C_{max} while T_{max} remained virtually unaffected resulting in a significant reduction in area under the curve. This interaction has not been observed in humans (Zhu *et al*., 1999).

Experiments in which rats were injected intra-peritoneally with a mixture of *trans*-anethole (100 mg/kg bw) and parathion-^{14}C (1.5 mg/kg) showed no significant effect of *trans*-anethole on metabolism and excretion of the insecticide. However, when rats were fed a diet containing 1% of *trans*-anethole for seven days and subsequently cell fractions from the livers of these rats were incubated for two hours with parathion-^{14}C, significantly less unchanged parathion (1.6%) was recovered compared to controls (12.5%). The data were interpreted as suggesting that feeding *trans*-anethole to rats for seven days induced the synthesis of parathion-degrading liver enzymes (Marcus and Lichtenstein, 1982).

Limonene was found to increase levels of reduced glutathione in mouse liver (Reicks and Crankshaw, 1993) and beta-myrcene was found to increase levels of specific subtypes of CYP450 in rat liver (De-Oliveira *et al*., 1997).

5.5.5. Fertility, pregnancy and lactation

There is pre-clinical evidence that acetone and ethanolic extract of fennel could impair fertility due its oestrogenic effects (see section 3.3.5 Reproductive and developmental toxicity), but no studies have investigated the effects of a water extract.

No fertility data in humans are available.

A water extract of fennel showed a dose-dependent teratogenic effect in mice (see non-clinical section 3.3.5 Reproductive and developmental toxicity), but the clinical relevance of the observed effect is not known.

There is evidence that *trans*-anethole is excreted in human breast milk (Hausner *et al*., 2008). Fennel has been traditionally used as galactogue. Foong *et al.* (2020) carried out a systematic revision of natural oral galactagogues, including fennel. Results were judged as uncertain about the magnitude of this effect because of substantial heterogeneity of the studies, imprecision of measurement methods and incomplete reporting; therefore, no definitive conclusion could be drawn on galactogue effects of fennel.

In a cohort study involving 630 pregnant women, collected data from mothers revealed that regular consumption of fennel during pregnancy (n=94 using fennel) can lead to shorter gestational age in women compared to non-users (Trabace *et al.*, 2015). No information on the amount of fennel was reported; in addition, the duration of fennel consumption was longer than that reported in the monograph.

In conclusion, safety during pregnancy and lactation has not been established; in the absence of sufficient data, the use of fennel is not recommended during lactation and pregnancy.

Finally, based on the HMPC "Public statement on the use of herbal medicinal products containing estragole" (EMA/HMPC/137212/2005 Rev 1 Corr 1*) that the use of estragole containing HMPs in in pregnant and breast-feeding women is not recommended if the daily intake of estragole exceeds the guidance value of 0.05 µg/kg bw.

5.5.6. Overdose

Cases of premature telarche have been reported in children under four years of age after several months of fennel herbal teas consumption. However, the data available is not relevant to be included in the monograph.

5.5.7. Effects on ability to drive or operate machinery or impairment of mental ability

No studies on the effect on the ability to drive and use machines have been performed.

5.5.8. Safety in other special situations

No data available.

5.6. Overall conclusions on clinical safety

No significant safety concern was identified during clinical trials and from post-marketing surveillance when fennel fruits were taken with the posologies supported by evidence of traditional use. However, in the general population exposure to estragole should be kept as low as practically achievable.

The use is not recommended in children under four years of age due to the lack of adequate data. The use in children between 4-12 years of age is not recommended if the daily intake of estragole exceeds the guidance value of 1.0 µg/kg bw.

Safety during pregnancy and lactation has not been established; in the absence of sufficient data, the use of fennel is not recommended during lactation and pregnancy. Importantly, the use of estragole containing HMPs in in pregnant and breast-feeding women is not recommended if the daily intake of estragole exceeds the guidance value of 0.05 µg/kg bw.

For further details related to estragole see "Public statement on the use of herbal medicinal products containing estragole" (EMA/HMPC/137212/2005 Rev 1).

Allergic reactions to fennel, affecting the skin or the respiratory system may occur. The frequency is not known.

6. Overall conclusions (benefit-risk assessment)

The traditional uses of fennel for "dyspeptic complaints such as mild, spasmodic gastro-intestinal ailments, bloating and flatulence", "for symptomatic treatment of minor spasm associated with menstrual periods" and "catarrh of the upper respiratory tract" are supported by long-standing use based on Article 16c of the Directive 2001/83/EC, but also by available data.

Clinical trials showing the efficacy of fennel as a smooth muscles spasmolytic remedy are limited and preliminary, but non-clinical pharmacological data show a relaxing effect of alcoholic extracts of fennel fruit on tracheal, ileal and uterine smooth muscles contracted by several contraction-inducing agents (i.e., acetylcholine, carbachol and histamine).

On the basis of long-standing use and experience, the HMPC identified the following indications for bitter fennel fruit and sweet fennel fruit: *"Traditional herbal medicinal product*

a) *for symptomatic treatment of mild, spasmodic gastro-intestinal complaints including bloating and flatulence;*
b) *for symptomatic treatment of minor spasm associated with menstrual periods;*
c) *used as an expectorant in cough associated with cold."*

The above recommended indications are exclusively based upon long-standing traditional use of fennel fruit and not on clinical trial data.

No other traditional medicinal uses of fennel within the European Union are supported by adequate data.

Method of administration: Oral use.

Duration of use

Because of the poor evidence of clinical trials, considering the lack of available safety data on long-term use of fennel preparations and due to the potential toxicity of estragole, a limit of two weeks is consistent with a self-medication indication, which is the case for traditional herbal medicinal products. In children, the use of fennel is for short-term in mild transitory symptoms only (less than one week).

If the symptoms persist during the use of the medicinal product, a doctor or a qualified health care practitioner should be consulted.

Anti-fertility effects have observed in pre-clinical studies only using acetone or ethanolic extract of fennel fruits, whereas water extract have not been studied. Estrogenic activity described for *trans*-anethole is not confirmed for aniseed alcoholic extracts on the basis of epidemiological data related to the common use of aniseed alcoholic beverages. Therefore it is not expected that *trans*-anethole, the major

constituent of anise and fennel essential oil could exert estrogenic effects when fennel is taken as a herbal infusion at the recommended posology.

Aqueous and methanolic extracts of fennel did not show any mutagenic activity in the Ames test using *Salmonella typhimurium* strains TA 98 and TA 100, with or without S9-metabolic activation, although several methodological shortcomings limit the validity of the results.

Results from studies carried out in the laboratory animals showed a weak mutagenic potential of anethole. However trans-anethole is reported as "generally recognised as safe" (GRAS) at the intake of 54 μg/kg bw per day) and the acceptable daily intake is 0-2 mg/kg bw.

Several studies have shown the carcinogenic effects of estragole in mice and rats (liver tumours) through a pathway including metabolic activation and DNA adduct formation; the same pathway is operative in human *in vitro* systems. There is general consensus that adduct formation is causally related to tumorigenesis, unless there are specific and biologically persuasive reasons to the contrary. Consequently, the mode of action for tumour formation is relevant for humans and the extrapolation of carcinogenicity to humans can be regarded as plausible (EMA/HMPC/137212/2005 Rev 1 Corr 1*). As a consequence, the HMPC has recently revised the "Public statement on the use of herbal medicinal products containing estragole" (EMA/HMPC/137212/2005 Rev 1 Corr 1*), concluding that "…the intake of estragole from HMPs in the general population should be as low as possible, which includes a short-time duration of use (maximum 14 days) and a discussion about the single/daily doses necessary according to the risk assessment relevant for the concerned HMP. For example, to reach or come as close as possible to the guidance value of 0.05 mg/person per day, the lowest dose should be consistently selected if ranges of single and daily doses are available from traditional use.".

Therefore, in the case of bitter and sweet fennel, for adults and adolescents only the lower dose of 1.5 g of fruits with 0.25 L of boiling water three times daily as a herbal tea has been included in the monograph. This corresponds to a daily dose of 4.5 g.

Similarly, for children between 4-12 years of age, only the lower dose of 1.0 g of fruits with 0.25 l of boiling water three times daily as a herbal tea has been included in the monograph. This corresponds to a daily dose of 3.0 g. However, the use is not recommended if the daily intake of estragole exceeds the guidance value of 1.0 μg/kg bw, unless otherwise justified by a risk assessment based on adequate safety data.

The use is not recommended in children under four years of age due to the lack of adequate data.

Considering the above-mentioned data and all the uses of fennel, it is concluded that human exposure resulting from short-term use of bitter and sweet fennel fruit as herbal tea, complying with the proposed specifications (referred to be normal conditions of use), is unlikely to pose any significant cancer risk for adults and adolescents. The same can be concluded for the use of fennel fruits as herbal tea in children between 4-12 years of age, if the daily intake of estragole does not exceed the guidance value of 1.0 μg/kg bw reported in the HMPC "Public statement on the use of herbal medicinal products containing estragole" (EMA/HMPC/137212/2005 Rev 1 Corr 1*).

However, each action from selection of cultivars and cultivation of the plant to the manufacture of herbal medicinal product containing fennel fruits, which could minimise the exposure of humans to estragole, should be recommended.

The European Union list entries on *Foeniculum vulgare* Miller subsp. *vulgare* var. *vulgare*, fructus and on *Foeniculum vulgare* Miller subsp. *vulgare* var. *dulce* (Mill.) Batt. & Trab., fructus have also been revised to reflect the changes made to the monographs. Only the lowest dose of the range supported

by the evidence of traditional use has been included. No safety concerns related to estragole are expected for adults and adolescents taking account the extraction rates following the preparation of the infusion and the short duration of use in the proposed indication, therefore the list entries are maintained.

Annexes

List of references